Inulin and Para-Aminohippuric acid: Determinants of Glomerular filtration rate and Renal blood flow following single intravenous bolus injection in man

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Declaration

The work described in this thesis has been conducted with the assistance and advice of those acknowledged on page iv.

I certify that this thesis has been composed by me alone.

J.A.N. McAuslane

ABSTRACT

Conventional methods for measuring glomerular filtration rate (GFR) and renal plasma flow (RPF) in man are based on the renal clearance of inulin and p-aminohippuric acid (PAH), determined by the laborious constant infusion method, with which urine collections are a major source of error. This thesis describes alternative simpler methods using single injection techniques with and without urine collections, using appropriate pharmacokinetic analysis and a specific HPLC assay for PAH and its acetylated metabolite (AcPAH).

The total body and renal clearances of inulin (70 mg/Kg) by the single intravenous injection technique were similar to the renal clearance by the standard constant infusion method, with sampling for the first two hours. After this time the total body and renal clearances of inulin declined progressively, following a single injection in normal subjects, and patients with renal impairment. The clearance of inulin was also dependent on plasma concentration during constant infusion at different plasma concentrations (35 to 187 mg/l). The renal clearance of creatinine remained constant during these studies and the fall in inulin clearance was therefore not due to renal changes in GFR. In normal subjects the critical plasma concentration below which the inulin clearances fell was about 100 mg/l, but was higher in patients with impaired renal function. The fall in inulin clearance at low plasma concentrations could be due to a lag in urinary excretion or saturable tubular reabsorption. Selective filtration of the high molecular weight polymers of inulin was unlikely. The kinetics were consistent with the saturable tubular reabsorption of inulin.

After a single injection of PAH (10 mg/Kg), the total body and renal clearances of PAH also fell after the first hour. The fall was even more dramatic than

observed with inulin. About 17 % of the dose of PAH was recovered in the urine as AcPAH. The renal clearance of AcPAH was greater than the PAH clearance and it rose after the first hour. The total body clearance of PAH overestimated the renal clearance of PAH during both constant infusion and after single injection, because of metabolism to AcPAH. The renal clearance (but not the total body clearance) of PAH varied directly with plasma concentrations during constant infusion over the range 4 to 25 mg/l. The renal clearance of AcPAH was always higher than the PAH clearance and was inversely related to plasma PAH concentration. The data were consistent with concentration-dependant renal acetylation of PAH. The renal clearances of PAH during constant infusion and for the first hour following single injection were similar.

Estimation of GFR and RPF by the single injection of inulin and PAH was quick, simple and easy. Using the single injection methods, renal function in healthy males was unaltered after administration of tenoxicam (a new non-steroidal anti-inflammatory drug) for 10 days.

At low plasma concentrations, the renal clearances of both inulin and PAH are plasma concentration dependent. Further studies are required to reassess the role of these compounds for measurement of the GFR and RPF in man.

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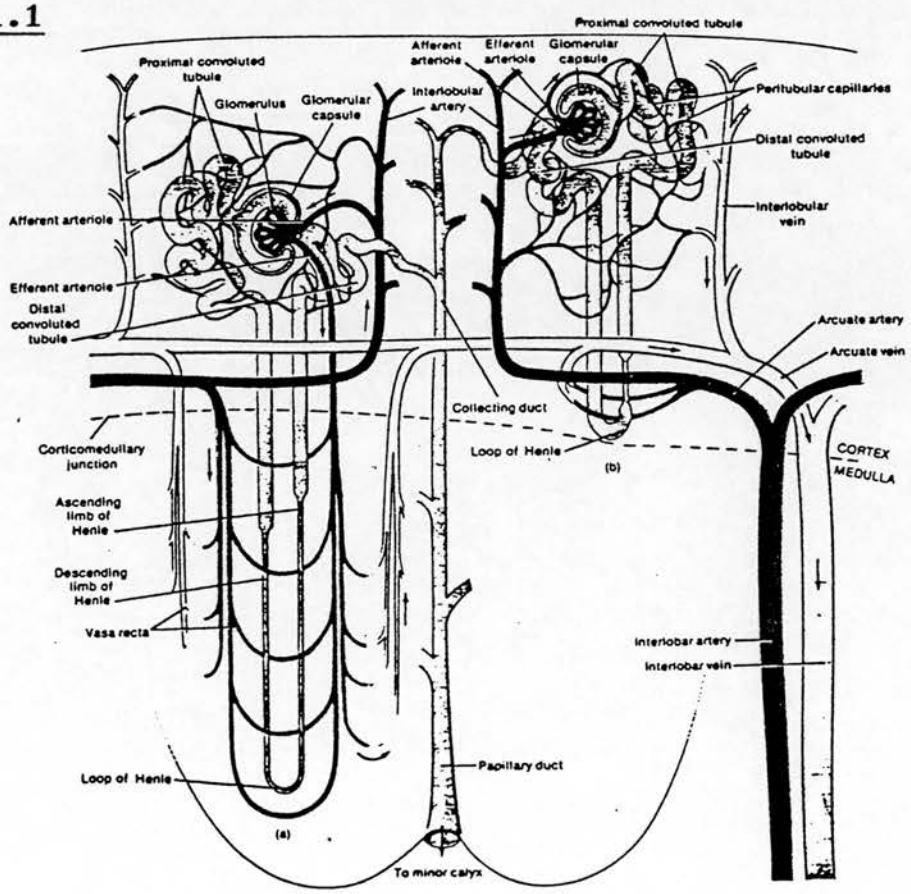
CHAPTER ONE

INTRODUCTION

STRUCTURE AND FUNCTION OF THE KIDNEY

The kidneys lie retroperitoneally on either side of the vertebral column. Each consists of an outer cortex and an inner medulla, leading into the renal pelvis and ureters. The functional unit of the kidney is the nephron, of which there are 1-1½ million per kidney. A nephron consists of the glomerulus and its capsule, a proximal convoluted tubule, the loop of Henle and the distal convoluted tubule. The nephrons are connected to collecting ducts, which open at the calyx of the renal pelvis, which in turn lead into the ureters and the bladder. Juxtamedullary nephrons have a long loop of Henle which extends deep into the medulla, while the cortical nephrons have short loops which do not penetrate far into the medulla (Fig 1.1).

Fig 1.1



Nephrons (a) Juxtamedullary. b) Cortical.
(from Tortora & Anagnostakos, 1984)

Ultrafiltration of blood plasma occurs at the glomerulus. The protein free filtrate enters the proximal tubule and essential substances such as glucose, electrolytes and amino acids are reabsorbed here, together with 80% of the filtered water. The proximal tubule cells can also secrete organic anions and cations by specific active transport mechanisms. The loop of Henle has a descending and an ascending limb, and its main function is the concentration of urine in conjunction with the medullary vasa recta and the collecting ducts (the 'counter-current multiplier'). The primary function of the distal tubule is facilitated reabsorption of Na^+ under the control of aldosterone, and the secretion of H^+, K^+ and NH_3^+. The collecting duct reabsorbs water under the control of antidiuretic hormone (Pitts, 1968; Guyton, 1986).

The kidneys receive their blood supply from the abdominal aorta via the renal arteries. The renal artery divides into anterior and posterior branches. The anterior branch supplies two thirds of the kidney. Segmental arteries divide into interlobular arteries, from which the specialised glomerular and peritubular capillary beds arise. The latter are in close relationship to the proximal and distal tubules, and long extensions supply the loop of Henle (the vasa recta) (Brenner et al, 1987).

The function of the kidneys can be summarised as filtration, reabsorption and secretion. They are responsible for the maintenance of water and electrolyte homoeostasis by varying the volume and composition of urine, and the elimination of waste products (eg urea, creatinine). The ability of the kidney to concentrate and reabsorb constituents of the filtrate coupled with its high blood flow (25% of cardiac output) and active metabolism, make it a common site of drug toxicity (Rush et al, 1984). Thus, it is essential to be able to

monitor kidney function in man and various techniques are available (Prescott, 1982a). Two important functions that are commonly measured are the glomerular filtration rate (GFR) and renal blood flow (RBF).

GLOMERULAR FILTRATION

The morphological studies of Bowman in 1842 provided the basis for the understanding of the process of glomerular filtration. He described the glomerulus as ideal for the separation of water from plasma, but thought that the urinary constituents were added by secretion (Pitts, 1968). Ludwig in 1844 proposed that the urine was formed by ultrafiltration at the glomerulus to give a protein free filtrate containing all urinary constituents, and that the filtrate was reduced in volume by reabsorption (Pitts, 1968). Direct evidence of glomerular filtration was subsequently obtained by Richards (1934b) using micropuncture techniques in amphibia, and urine was shown to be derived from ultrafiltrate of plasma. This process was confirmed in small mammals by Walker et al,(1941).

Glomerular structure

The glomerular capsule is indented and envelops a tuft of capillary loops, which spring from the afferent arteriole and end in the efferent arteriole. The glomerular capillary wall consists of three specialised layers. On the blood side of the capillary lies, a layer of endothelial cells separated by fenestrations or gaps (500-1000 A°). These endothelial cells lie on an acellular basement membrane composed of acidic glycosaminoglycans and a supporting matrix of collagen fibres. On the urinary side of the basement membrane, lie a layer of specialised epithelial cells (podocytes) displaying a complex pattern of interdigitating foot processes (pedicils) (Ryan, 1986).

Glomerular filtration dynamics

Filtration at the glomerulus is the first step in the production of urine and approximately 180 litres a day are filtered. The driving force for glomerular filtration is the hydraulic pressure gradient across the capillary membrane. The glomerular filtration rate (GFR) is dependant upon the balance of hydraulic (P), and oncotic (II) pressure (Starling's forces) between the capillary and the tubule. The oncotic pressure is derived from the presence of proteins in the capillary which are absent in the tubule fluid, therefore the tubular oncotic pressure is essentially zero. Two other important determinants are the intrinsic permeability of the capillary wall (k), and the available surface area (A) for filtration. Thus for a single nephron, the GFR is determined by the relationship shown in equation 1 (Brenner & Humes, 1977; Baylis, 1986).

$$GFR = kA\ (\Delta P - \Delta II) \qquad \text{(Equation 1)}$$

(ΔP & ΔII = mean difference in the hydraulic and oncotic pressures between the capillary and tubule)

Alteration of the different components of equation 1, will have an effect on the single nephron GFR as shown in Table 1.1. The glomerular capillary hydraulic pressure is kept constant through a balance between the afferent and efferent vascular resistance, and it is always higher than the capillary oncotic pressure.

The GFR is maintained relatively constant over a wide range of arterial blood pressures due to autoregulation by intrinsic vascular responses of the afferent and efferent arterioles. Vasoactive agents involved in this process include angiotensin II, catecholamines (noradrenalin, dopamine), antidiuretic hormone and prostaglandins. They act by modifying the capillary resistance, capillary permeability and surface area for filtration (Dworkin et al, 1983; Baylis, 1986; Lote & Haylor, 1986).

TABLE 1.1

A) **Factors contributing to an increased GFR**	**Examples**	**Reason**
1) increased mean hydraulic pressure	hypertension	ΔP
2) reduced plasma protein concentration	malnutrition	$\Delta \Pi$
3) increased glomerular plasma flow rate	Anaemia, pregnancy	$\Delta \Pi$

B) **Factors contributing to decrease GFR**		
1) reduced hydraulic pressure	hypotension	ΔP
2) increased plasma protein concentration	hyperglobulinemia	$\Delta \Pi$
3) reduced glomerular plasma flow rate	shock, dehydration	$\Delta \Pi$
4) reduced permeability properties of the capillary wall	glomerulonephritis	kA

(adapted from Brenner et al, 1987)

Glomerular filtration barrier

The glomerular capillary is very permeable to water, but the ultrafiltrate normally contains very little plasma protein or other macromolecules. The selectivity of the glomerular barrier for filtration of large molecules, is determined by their size, charge and configuration (Deen et al, 1983). Molecules which have an effective radius of 1.4 nm or less, are freely filtered (fractional clearance = 1). As the effective radius increases with increasing molecular size, the fractional clearance diminishes progressively. It approaches zero for serum albumin (effective radius 3.55 nm, MW 68000), but with neutral dextrans, the fractional clearance does not approach zero until the effective radius exceed 4.2 nm, possibly because of charge selectivity. Albumin is anionic, and anionic dextran sulphate shows a reduced fractional clearance compared to neutral dextrans of the same effective radius. In contrast, the fractional clearance of cationic dextran is increased. This charge selectivity is due to the presence within the capillary wall of fixed negative

charges which are found in all regions of the capillary, and are thought to impede the filtration of anionic macromolecules (Bohrer et al, 1978; Brenner et al, 1978). The higher fractional clearances of neutral dextrans could also be related to their linear polymeric nature. These polymers are loose, randomly coiled, hydrated spheres in free solution. These can unfold in response to deforming forces of pressure and become elongated, passing through the capillary wall by reptation (wriggling end on through the interstices). Such unfolding cannot occur with globular proteins such as albumin because of the firm, internal cross-linking or folded polypeptide chains (Ryan, 1986).

MEASUREMENT OF GLOMERULAR FILTRATION RATE

The measurement of GFR is important for the study of changes induced by drugs, disease or physiological factors. Clinically, it is used to assess renal function in patients with nephro-urological disorders. Dosage requirements for drugs which are excreted by the kidney, and have a low therapeutic ratio, are based on the GFR. If the renal clearance of a substance is greater than the GFR, it must also be secreted, if it is less, then reabsorption may be taking place (Brochner-Mortensen, 1985; Schuster & Seldin, 1985).

The single nephron glomerular filtration rate can be measured directly in animals using micropuncture techniques, but this is not possible in man. The GFR can be estimated for all functional glomeruli by measuring the clearance of a suitable substance excreted by the kidney (Levinsky & Levy, 1973). Clearance relates the concentration of a substance to its rate of elimination (Rowland & Tozer, 1980) :-

rate of elimination = clearance x concentration

If an amount of such a substance is found in the urine (Ax), then it must have been cleared from a volume of plasma containing that amount. Thus, the renal

clearance (Clr) can be conceived of as a proportionality constant, relating the urinary excretion rate (dAx/dt), to its plasma concentration (Cp). So it can be defined as the virtual volume of plasma completely cleared of that substance per unit time, and the renal clearance can be estimated by comparing the amount of substance excreted over time, to its concentration in plasma (Gibaldi, 1984).

$$Clr = \frac{dAx/dt}{Cp} \qquad \text{(Equation 2)}$$

In practice, the renal clearance of a substance x (Clx) is calculated as follows :-

$$Clx = \frac{Ux\ V}{Px} \quad ml/min \qquad \text{(Equation 3)}$$

where Px and Ux are the concentrations of x in the plasma and urine respectively, and V is the urine flow rate in ml/min. Alternatively, the renal clearance can be calculated from the slope of a plot of the urinary excretion rate of a compound against the plasma concentration, provided that the renal clearance is independent of the plasma concentration (Tucker, 1981). The renal clearance can also be determined if the right hand side of equation 2 is intergrated with respect to time (Notari, 1987) :-

$$Clr = \frac{\text{AMOUNT EXCRETED}}{\text{CORRESPONDING AUC}} \qquad \text{(Equation 4)}$$

where the AUC is the area under the plasma concentration-time curve for the corresponding time period. The AUC is usually measured by the trapezoidal method (Tucker, 1981).

These equations apply irrespective of the handling of a substance by the kidney (ie, whether it is filtered at the glomerulus, secreted or reabsorbed by the tubules). It is therefore necessary to use a substance which is cleared by the mechanism which represents the

kidney function to be measured. For measuring GFR, an ideal substances should have the following characteristics (Smith, 1951) :-

It should be -

1) Non-metabolised and freely filtered by the glomerulus.
2) Neither secreted nor reabsorbed by the tubules.
3) Neither bound to plasma proteins or stored in the kidney.
4) Physiologically inert.

For such a test compound the renal clearance equals the GFR.

Compounds used for measurement of the GFR

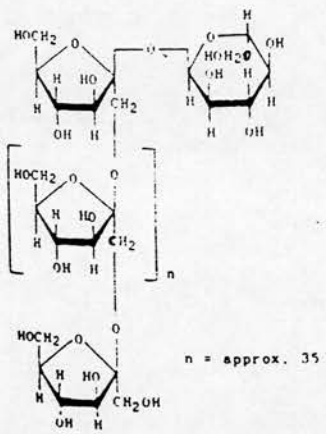
The clearance of urea was originally used as an index of kidney function by Addis (1917), who showed that the ratio of the rate of urea excretion per hour to the blood urea concentration was constant, at maximal urine flow rates. However, the rate of urea excretion is dependent on the urine flow rate (Austin et al, 1921). Moller, McIntosh and Van Slyke (1929) introduced the term 'clearance' in connection with urea excretion. Rehberg (1926) chose creatinine as the index substance because it was the urinary constituent which was concentrated most, relative to plasma. Joliffe and Smith (1931) extended the concept of clearance to creatinine, and this principle is now widely used to define the removal of substances from the body. Creatinine and urea are unsatisfactory markers, because they are subject to tubular secretion and reabsorption respectively.

Richards et al, (1934b) and Shannon and Smith, (1935) compared the clearances of inulin, xylose and creatinine and found that inulin appeared to best fit the requirements for the ideal substance to measure GFR. It has since become the universally accepted standard marker (Schuster & Seldin, 1985).

Inulin

Inulin was first isolated from artichoke tubers by Rose in 1804 (McDonald, 1946). It is a polyfructosan (polysaccharide), which upon acid hydrolysis yields D-fructose as the main product. It is insoluble in cold water, but dissolves readily on heating to 80°C. Supersaturated solutions may stay clear for 3 to 5 days, before visable precipitation occurs (Phelps, 1965). Inulin consists of an average of 30 fructofuranose subunits in an unbranched chain which terminates with a nonreducable D-glucose unit (Fig 1.2) (Middleton, 1977).

Fig 1.2



The structure of Inulin

The average molecular weight of inulin is about 5200, (Levinsky & Levy, 1973) with an average range of 4000 to 7200, depending on the method of determination, and the time of year that the tubers are harvested (Berglund, 1965; Phelps, 1965). However, inulin is not homogeneous, and it consists of a complex mixture of polymers, with a wide range of molecular weights up to 15,000 (Bassir, 1956; Mogensen, 1968). Commercial inulin is composed of two fractions, an alkali labile fraction of low molecular weight, which is hydrolysed by hot

dilute alkali and an alkali stable fraction of high molecular weight. The proportions of these fractions vary from 1:12 to 2:1, in inulin from different sources (Cotlove, 1954; Andreucci, 1978). However, their renal clearances are reported to be similar. (Cotlove, 1954; Walser et al, 1955).

The solvated inulin molecule is cylindrical, with a semilength of 25 A°, a radius of 10 A° (Middleton, 1977), and a diffusion coefficient of about 1.33 x 10^{-6} cm^2/sec. This is consistent with a molecular weight of about 15,000, and this discrepancy has been attributed to an elongated configuration (Bunim et al, 1937; Phelps, 1965).

Inulin clearance as a measure of glomerular filtration

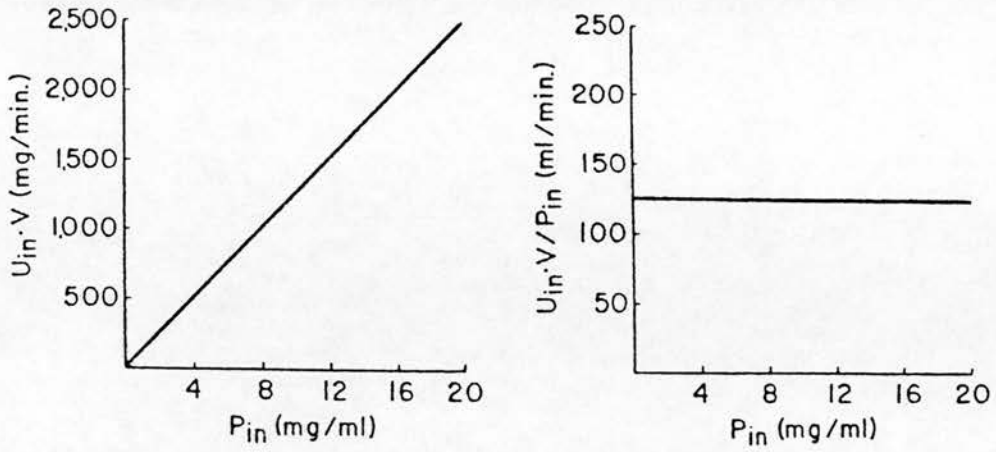
Homer Smith (1951) has given a detailed account of the validation of the inulin clearance as a measure of GFR in animals and man. The urinary excretion rate of inulin, is said to be proportional to its plasma concentration over the wide ranges of 50-400 mg/100 ml, (Shannon & Smith, 1935) and 5-90 mg/100 ml (Miller et al, 1940). Thus, the renal clearance of inulin appears to be independent of the plasma concentration (Fig 1.3a & 1.3b). This suggests that inulin is freely filtered at the glomerulus, and is neither reabsorbed or secreted by the tubules. The renal clearances of substances which are secreted (e.g. p-aminohippuric acid) or reabsorbed (e.g. glucose) by the renal tubules approach the inulin clearance when these mechanisms are saturated or inhibited with phlorizin respectively.

Inulin is not bound to plasma proteins (Shannon, 1934; Richards et al, 1934a) or metabolised (Smith, 1951). It is nontoxic and inert, and has been given intravenously in man for over 40 years in doses as high as 160 g without reported ill effects. The only severe reactions to inulin, have been associated with pyrogen contamination (Shannon & Smith, 1935; Materson, 1971).

FIG 1.3

The relationships between the rate of excretion (1.3a) and clearance (1.3b) of inulin to plasma inulin concentration. (adapted from Pitts, 1968) Uin and Pin represent the concentration of inulin in urine and plasma respectively. V is the urine flow rate

1.3 a) 1.3 b)



Inulin does not affect the renal clearance of other substances, (eg urea, xylose) and it is rapidly excreted in the urine with quantitative recovery (Shannon & Smith, 1935; Schwartz et al, 1949; Ladefoged, 1969). Inulin distributes slowly through the extracellular water, and it has been used to determine the extracellular space. Its volume of distribution is 13-16 % of the body weight (Schwartz et al, 1949; Ladegaard-Pedersen, 1972). It is thought that a small, but insignificant amount of inulin is excreted in the bile (Smith, 1951).

The evidence that inulin is cleared at the rate of glomerular filtration has been questioned by Berglund (1965). He found that the renal clearance of polyethylene glycol (PEG 1000) in the rat was greater than the inulin clearance, and suggested that the filtration of inulin at the glomerulus was restricted.

In man, the renal clearance of inulin has been reported to decline, as its plasma concentration falls (Ferguson et al, 1950; Barnard et al, 1955). However a decline in the renal clearance of inulin after a single injection was not observed by Alving & Miller (1940), or during clearance measurements at different plasma concentrations of inulin, produced by stepping up or down the infusion rate (Kennedy & Kleh, 1953; Cole et al, 1972). No evidence supporting either tubular reabsorption of inulin or plasma concentration dependent clearance, was found. In the rat, micropuncture studies have shown that inulin is filtered freely at the glomerulus, and urinary recovery is quantitative, after an injection into the tubular fluid. Inulin was not detected in the blood or urine from the contralateral kidney (Gutman et al, 1965; Harris et al, 1974). Mogensen (1968) infused inulin from different commercial sources into normal subjects, and patients with renal impairment. Sephadex gel filtration was used to separate fractions of differing molecular weights in plasma and urine, and the elution curves were identical, indicating unrestricted filtration. Similar findings have been reported in preterm babies (Coulthard & Ruddock, 1983).

Overall, the evidence strongly suggests that inulin is cleared at the rate of glomerular filtration, and it has become universally accepted as the standard marker for measurement of GFR in both man and animals (Pitts, 1968; Schuster & Seldin, 1985).

Polyfructosan-S

This is an inulin analogue which was first isolated from the bulbs of red squill by Schmeideberg in 1879 (Nitsch et al, 1979). It is a β-D-fructan of the inulin type, with branching at position 6. The molecular weight distribution ranges from 800 to 16,000, with an average of 3000 (Nitsch et al, 1979). Polyfructosan-S was introduced as an alternative to inulin because it is very

water soluble and alkali stable (Materson, 1971; Nitsch et al, 1979). Its renal clearance does not differ significantly from that of inulin (Mertz, 1963; Levinsky & Levy, 1973; Muller-Suur et al, 1983). Polyfructosan-S is assayed in the same way as inulin after hydrolysis to fructose (Materson, 1971), and it has been used to measure GFR in both animals and man (Falbriard & Zender, 1964; Favre et al, 1968; Svenninigsen, 1975; Muller-Suur et al, 1983).

CLEARANCE TECHNIQUES - INULIN

Constant infusion

Smith and his colleagues developed the standard method for determining the GFR by measuring the renal clearance of inulin at a constant plasma concentration. An intravenous priming dose of inulin is administered to achieve the required plasma concentration, and this is followed by a continuous infusion, to maintain that concentration. An equilibrium period of about one hour is required to achieve steady state plasma concentrations after which, accurately timed urine samples are collected every 30 minutes for 3 hours. A plasma sample is taken at the midpoint of each urine collection period. The inulin clearance for each period is calculated according to equation 3, and the mean value is taken as the GFR (Smith, 1951). This method can be accurate, but it depends critically on carefully timed urine collections, and complete bladder emptying. This requires bladder catheterisation and fluid loading to ensure an adequate urine flow.

The normal mean values for inulin clearance compiled by Smith (1951), and Wesson (1969), from numerous investigators using the above technique, are 127-130 ml/min/1.73m^2 for males, and 118-120 ml/min/1.73m^2 for females, aged 20 to 40 years. The inulin clearance is about half the adult rate in the newborn, and it reaches

adult levels by about 2 years of age. After 40 years of age, there is a steady decline, and the decline in the inulin clearance in the eighth decade is half that at 20 years (Davies & Shock, 1950a; Schuster & Seldin, 1985).

The normal range of inulin clearance is 72-176 ml/min/1.73m^2 in males, and 81-137 ml/min/1.73m^2 in females (Smith, 1951). Sources of variation include experimental and analytical errors, and physiological variation in the GFR. Experimental variability on the day of measurement may produce a standard deviation of about 9% (Kennedy & Kleh, 1953). Zender et al, (1968), found an intra-assay coefficient of variation of 1.73%, with an error in the measurement of urine flow rate of 7.1%. Urine collection is therefore an important source of error. Davies & Shock (1950b) found greater variation from day to day than in sampling periods on the same day, and a day to day difference of 20 ml/min, represented a 95% chance of a significant change in GFR. Physiological influences on GFR include exercise, pregnancy and stress. There is also a diurnal variation in the inulin clearance, with the lowest values at 4.00 am and the highest at 2.30 pm. Thus, clearance measurements should be made at the same time of day (Wesson & Lauler, 1961). A protein load increases the inulin clearance in animals, and this also occurs in man, with increases of up to 22% (Pullman et al, 1954).

Inulin clearance in disease

Measurement of the GFR has been described as "the single best assessment of the severity and evolution of renal failure" (Editorial, 1967), and the inulin clearance has been widely used to measure the GFR in patients with progressive renal failure (Skov, 1970; Hagstam et al, 1974; Schnurr et al, 1980). However, impairment of glomerular function may restrict free filtration of inulin, and leakage may occur in the presence of tubular damage (Smith, 1941). The former is

unlikely as the renal clearance of hexitols, mannitol and inulin are identical in patients with pre-eclamptic renal failure and glomerulonephritis (Smith, 1941; Earle et al, 1944). The inulin clearance is considered a reliable index of glomerular filtration in patients with diseased glomeruli and the nephrotic syndrome (Carrie et al, 1980). The reliability of the inulin clearance has been questioned in transplant patients and donors (Rosenbaum et al, 1979), but the inulin, creatinine and iothalamate clearances were similar in children receiving renal transplants (Mak et al, 1983). Transtubular reabsorption of filtered inulin may occur in experimental disease, tubular damage and nephrotoxicity induced by agents such as mercury (Bank et al, 1967; Biber et al, 1968). Thus, in patients with renal impairment, there are still potential pitfalls in the interpretation of the inulin clearance as a measure of GFR.

Disadvantages of the constant infusion method for measuring inulin clearance

This technique has come to serve as the reference for other methods (Truniger et al, 1968). However, it is tedious and cumbersome not only for the patient, but also the investigator. Errors and problems arise because of:-

1) INCOMPLETE BLADDER EMPTYING
2) IMMOBILISATION OF PATIENTS
3) DELAY TIME

1) INCOMPLETE BLADDER EMPTYING

The greatest single source of error is in the collection of urine, and complete bladder emptying is necessary for accurate estimations of GFR. In normal, healthy young subjects, 1-2 ml of urine remain in the bladder after micturition, so that at normal flow rates of 1-2 ml/min, the error in a 30 minute collection

period could be as much as 7% (Tucker, 1981). Accurate collections require bladder catheterisation with washouts, but this carries the risk of urinary tract damage and infection, and it is unpleasant. Errors due to incomplete bladder emptying can be reduced by :-

A) Water diuresis, as the residual volume may then be negligible, compared with the total volume excreted. This in turn is a disadvantage, as fluid loading places the kidney in an abnormal physiological state.

B) An increased period of collection. This in turn prolongs the infusion period.

2) IMMOBILISATION OF THE PATIENT

The need for a continuous infusion to steady state requires immobility for 3 to 4 hours. Also, the need for at least four short urine collection periods with catheterization or fluid loading, makes the procedure a trial, even for healthy subjects.

3) DELAY TIME

Its takes time for a substance to be filtered and pass down the tubule, before it arrives in the bladder. This delay time has two components, the time taken to pass down the nephron, and the time for the new urine to mix with that previously secreted (Nosslin, 1965). In practice, this means that the rate of excretion lags behind that appropriate for the plasma concentration at a given moment. Thus, ideally the plasma should be sampled between 2.5 and 5 minutes before the midpoint of the urine collection period (Materson, 1971). In patients with normal renal function, a delay time correction factor can be determined from the urine flow rate (Nosslin, 1965).

Due to these problems, the constant infusion method for estimating GFR is impractical for routine clinical use, and today it is employed mainly for research. The need for urine collection and prolonged intravenous

infusions, can be avoided by constant infusion without urine collection and, single intravenous injections with and without urine collection.

Constant infusion without urine collection

In this method, measurement of the plasma clearance or total body clearance (TBC) of a test substance is based on the assumption that, if steady state plasma concentrations are achieved, the rate of infusion is exactly equal to the rate of elimination. If, as with inulin, the kidney is the only route of elimination the rate of infusion equals the rate of excretion. The GFR is equal to the rate of excretion divided by the plasma concentration. Therefore, the total body clearance can be determined simply by dividing the rate of infusion, by the steady state plasma concentration (Css) (Notari, 1987).

$$TBC = \frac{\text{rate of infusion}}{Css} \qquad \text{(Equation 5)}$$

Earle and Berliner (1946), introduced this technique with inulin to obviate the need for urine collections. The results agreed well with values obtained using the standard constant infusion method (Berger et al, 1948; Cole et al, 1972; Schnurr et al, 1980). However, it is not possible to establish whether steady state exists, and the total body clearance of inulin determined this way may be an overestimate of the simultaneously determined renal clearance (Ladefoged, 1969; Rose, 1969). These differences were attributed to incomplete bladder emptying. The method has been reported to be the most accurate in newborn babies, in which constant infusions for 24 hours give the most reliable results (Coulthard, 1983).

The advantage of this technique is its simplicity. All that is required is the infusion of inulin, and the taking of one blood sample. The main disadvantage is

that steady state plasma concentration has to be achieved, and with normal renal function this takes approximately 4 x the half life (Gibaldi, 1984), i.e. about 5 hours for inulin. A much longer period is required in patients with impaired renal function although, this can be reduced somewhat with the use of a loading dose. The patient is immobilised for a prolonged period, and the method is of little use in dynamic studies as, small changes in GFR will not be reflected quickly by changes in the plasma concentration (Materson, 1971; Cole et al, 1972).

Single injection without urine collection

This method also measures the total body clearance (TBC) of a substance. If excretion is limited to the kidney, and is solely by glomerular filtration, then the TBC will equal the GFR (Wilkinson, 1987). After a single intravenous bolus injection of a GFR marker such as inulin, the plasma concentrations fall in a curvilinear manner when plotted logarithmically against time. An initial rapid decline in plasma concentration is due to both distribution and elimination, and is followed by a slower linear terminal phase due solely to elimination (Notari, 1987). The clearance is calculated from the plasma decay curve by relating it to the ratio of the dose administered and, the area under the plasma concentration-time curve (AUC) (Wilkinson, 1987). Plasma concentration-time curves have been analysed and the clearance calculated by three main pharmacokinetic methods.

1) One compartment model (Slope-intercept method)
2) Open 2-compartment model
3) Model-independent analysis

1) In the one compartment model, the slope of terminal linear elimination phase is extrapolated back to zero time to give the initial plasma concentration. It is incorrectly assumed that the inulin in plasma

equilibrates rapidly with that in the extracellular fluid. The terminal slope is taken to reflect removal by GFR alone, (Newman et al, 1944) and the clearance is calculated using the following formula:-

$$TBC = \frac{Dose\ K1}{B} \qquad \text{(Equation 6)}$$

Where K1 is the slope of the regression line and B is the zero time concentration obtained by back extrapolation of the terminal linear slope to the Y axis. This method requires few blood samples and is very easy. However, the model is quite inappropriate as the whole phase of distribution is ignored (Truniger et al, 1968; Cohen, 1974), and it seriously overestimates the TBC.

Although it has been claimed that the one compartment method using data obtained after a bolus injection of inulin correlates well with the standard inulin clearance, it is inevitable that the clearance will be overestimated (Rosenbaum et al, 1973). Barnett (1940) used a similar method and found a good correlation between the slope of the terminal linear phase, and the standard inulin clearance. A similar approach has been used with Polyfructosan-S, and the results correlated well with the standard clearance. (Falbriard & Zender, 1964; Zender & Falbriard, 1967; Favre et al, 1968).

2) In the open 2-compartment model it is assumed that the dose is injected into one central compartment, and that it diffuses freely into a second peripheral compartment with elimination from the central compartment (Sapirstein et al, 1955). This is a commonly used model for pharmacokinetic analysis. In practice, the log plasma concentration-time curve is the resultant of two simultaneous exponential processes, which can be separated by curve peeling. The elimination phase is derived from the terminal linear part of the graph which is extrapolated back to the Y axis, giving a zero time

concentration (B). The half life ($t_{\frac{1}{2}\beta}$) for this phase, is calculated from the slope of the line using the following relationship (Rowland & Tozer, 1980):-

$$t_{\frac{1}{2}} = \frac{\text{Log}_{10}\ 2}{\text{Slope}} \qquad \text{(Equation 7)}$$

The rate constant for elimination is calculated by dividing the natural log of 2 by the half life. The early values of the original plasma concentration-time curve exceed those of the back-extrapolated line, and the differences yield a series of residuals. These are plotted as a second straight line with an intercept on the Y axis (A), and a half life ($t_{\frac{1}{2}\alpha}$) (Fig 1.4). The sum of A + B is equal to the maximum concentration at zero time (Co).

This biexponential curve can be described using the following equation:-

$$Co = Ae^{-\alpha t} + Be^{-\beta t}$$

where α and β are the rate constants for distribution, and elimination respectively. The total body clearance (TBC) can be derived from the following relationship between the area under the plasma concentration-time curve (AUC), and the administered dose:-

$$TBC = \frac{DOSE}{AUC_{\ 0-\infty}} \qquad \text{(Equation 8)}$$

If the concentration-time curve is biexponential (Notari, 1987):-

$$AUC\ 0-\infty = A/\alpha + B/\beta \qquad \text{(Equation 9)}$$

therefore:-

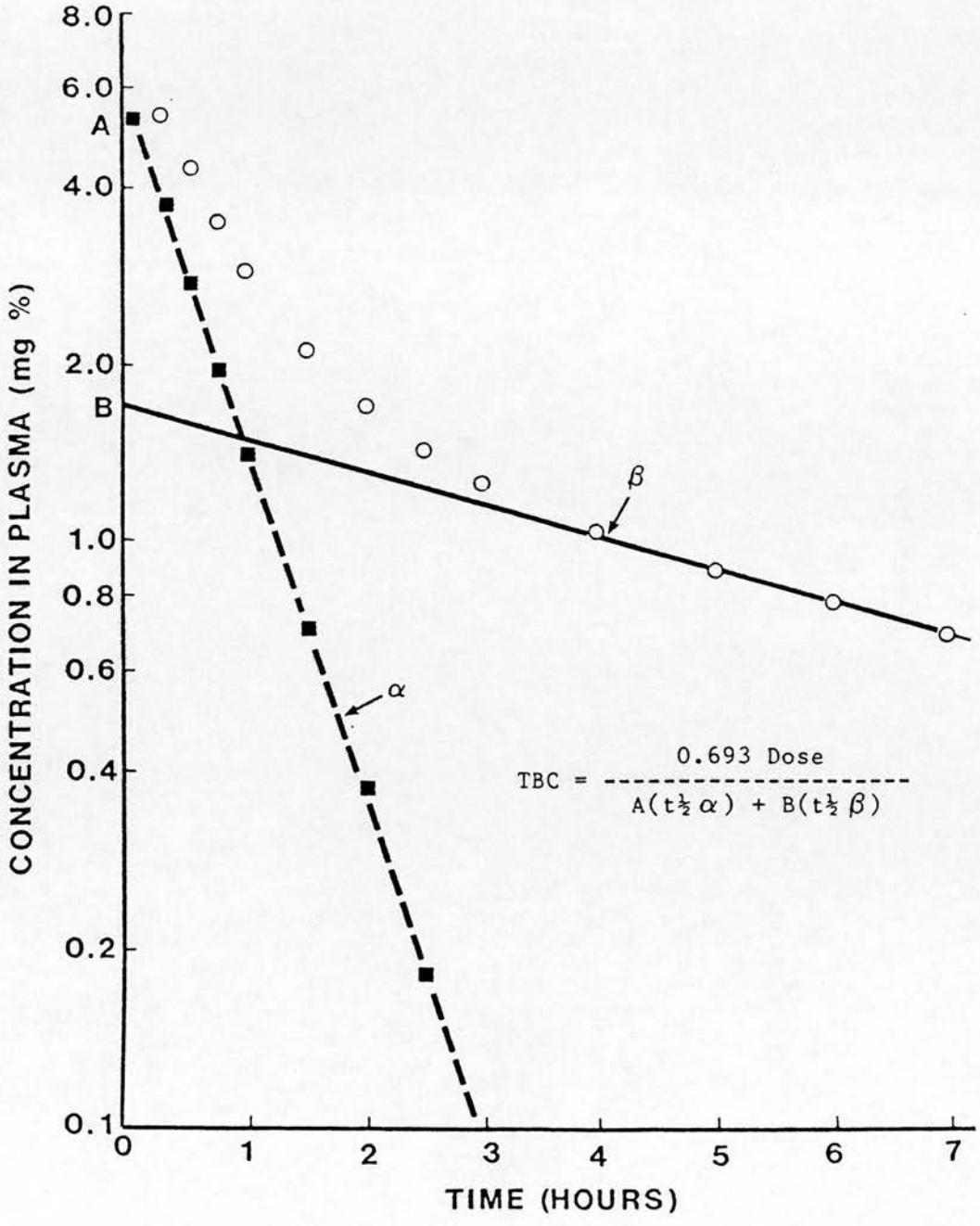
$$TBC = \frac{DOSE}{A/\alpha + B/\beta} = \frac{DOSE\ \alpha\ \beta}{A\beta + B\alpha} \qquad \text{(Equation 10)}$$

This can be rearranged to give:-

$$TBC = \frac{0.693\ Dose}{A(t_{\frac{1}{2}\alpha}) + B(t_{\frac{1}{2}\beta})} \qquad \text{(Equation 11)}$$

Fig 1.4

Two compartment model analysis.



This is the equation for TBC based on a two compartment model (Cohen, 1974). The $t_{\frac{1}{2}}\alpha = 0.693/\alpha$ and the $t_{\frac{1}{2}}\beta = 0.693/\beta$ where 0.693 is the natural logarithm of 0.5. In reality, the situation is often more complex with distribution into more than two compartments. In such circumstances, the calculated clearance tends to be overestimated (Chantler et al, 1969; Svenningsen, 1975). The total body clearances of inulin and polyfructosan-S have been measured using this model (Broberger, 1973; Silkalns et al, 1973; Svenningsen, 1975; Fawer et al, 1979; Muller-Suur et al, 1983). It accounts for the distribution of inulin after a bolus injection, and good correlations were reported with clearances obtained by the standard constant infusion method.

3) Model independent analysis requires no assumption of the number of compartments or that the indicator is in distribution equilibrium. The general form of the plasma decay curve is not important, but it is assumed that all the administered dose is completely eliminated (Gibaldi, 1984).

As shown above:-

$$TBC = \frac{DOSE}{AUC\ 0\text{-}\infty} \qquad \text{(Equation 8)}$$

In practice, it is not possible to measure the entire plasma concentration time curve, and extrapolation from the last data point to infinity is necessary to calculate the area of the final "tail". The final AUC to infinity can be calculated by dividing the last plasma concentration time point (P(t)), by the terminal elimination constant (β).

$$AUC\ t\text{-}\infty = \frac{P(t)}{\beta} \qquad \text{(Equation 12)}$$

The curve may also be resolved into monoexponetial functions by curve peeling, and the total area calculated as the sum of the areas under each, i.e. for a two compartment model, the AUC = $A/\alpha + B/\beta$ (see above). Thus,

the appropriate compartmental analysis of the plasma concentration time curve and model independent pharmacokinetic analysis reduce to the same terms, and are equivalent. The advantage of the model independent method is no assumptions have to be made. If the compound is eliminated completely by renal excretion, total body clearance equals renal clearance.

Either method provides an absolute means of measuring the GFR provided that 1) sufficient number of plasma samples are taken to define the early part of the curve, 2) sampling is continued until the final slope is attained and 3) the inulin clearance remains constant during the sampling period. These methods are accepted as the most accurate for measuring the TBC of a substance after a single injection (Nosslin, 1965; Ladegaard-Pedersen, 1972; Hall et al, 1977; Brochner-Mortensen, 1985). The total body clearance of inulin calculated by the model independent method has been reported for inulin in normal subjects (Ladegaard-Pederson, 1972), and in patients with a single kidney (Rehling et al, 1984). The latter author, also measured the renal clearance of inulin at the same time and showed it to be significantly less than the total body clearance.

Single injection with urine collection

Early investigators calculated the inulin clearance from the terminal slope of the plasma concentration-time plot using equation 3. It was assumed, that distribution equilibrium had occurred between plasma and extracellular water, and that the system conformed to a one compartment model (Alving & Miller, 1940). This is not the case. The renal clearance is more correctly estimated from the amount excreted in a given time divided by the corresponding AUC (equation 4) (Notari, 1987). This gives a time averaged value for the renal clearance, but urine can be collected over a longer period of time. However, collections must be complete. This technique is less

sensitive to changes in renal clearance with time than the constant infusion method (Tucker, 1981; Wilkinson, 1987). If venous blood is sampled, the renal clearance may underestimate the true clearance if there are significant differences between the arterial and venous plasma concentrations (Brochner-Mortensen, 1985).

Alving and Miller (1940) introduced this method for the clinical measurement of the inulin clearance. Robson et al, (1950), doubted whether equilibration occurred rapidly after a single injection and devised a formula to correct for this, reporting good agreement with the standard constant infusion method in 19 subjects (ratio 1.04 $\pm$ 0.14). The same group also reported that the renal clearance of inulin falls progressively after a single injection, and suggested that inulin is reabsorbed by the tubules (Ferguson et al, 1950). Some workers have reported a similar decrease in clearance (Josephson & Lindahl, 1943; Laake, 1954; Barnard et al, 1955), but others have not (Alving & Miller, 1940; Carrie et al, 1980). Denneberg et al, (1961), reported that the inulin clearance can be calculated with reasonable accuracy by dividing the 24 hour urine recovery of inulin, by the 0-2 hour plasma curve extrapolated to infinity. The advantages and disadvantages of each technique for measuring the GFR are summarized in Table 1.2.

Problems associated with single injection techniques

Objections to the use of single injection techniques are related to errors associated with failure to take distribution kinetics into account, adequate sampling, arterial venous differences and the delay time (Smith, 1951). Some of these objections have been minimised by a better understanding and application of pharmacokinetic principles.

VOLUME OF DISTRIBUTION

Early investigators incorrectly assumed that inulin

Table 1.2

THE ADVANTAGES AND DISADVANTAGES OF THE TECHNIQUES USED FOR THE MEASUREMENT OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL BLOOD FLOW

TECHNIQUE	ADVANTAGE		DISADVANTAGE
CONSTANT INFUSION			
A) with urine collection	reference method accurate & precise at low GFR useful: in dynamic studies when GFR not constant research tool		time consuming immobilisation of patient catheterization fluid loading not clinically practicable
B) without urine collection	no urine needed		immobilisation of patient for long periods steady state plasma concentration necessary
SINGLE INJECTION TECHNIQUE			
without urine collection			
A) one compartment model	simple to do	no urine needed simple	overestimates the clearance
B) two compartment model	most used model	rapid patient comfort	overestimates the clearance if the model is wrong
C) Model independent	requires no assumptions	practical	numerous blood samples needed to define curve
with urine collection	measures renal clearance directly		needs urine collection

is diluted instantaneously in the whole volume of distribution. However, equilibration between plasma and extracellular fluid takes a considerable time, as shown by the progressive increase in the distribution volume (Levinsky & Levy, 1973). In the 2 compartment and model independent analysis, the distribution phase is taken fully into account.

ADEQUATE SAMPLING PERIOD

The terminal elimination phase must be accurately defined for measurement of the total AUC to derive the total body clearance. In practice, this dictates the sampling period and, if this is too short, the AUC to infinity will be underestimated, and the total body clearance overestimated (Chantler et al, 1969). The sampling time may have to be greatly increased in patients with renal disease or severe oedema and ascites, due to an increased volume of distribution (Brochner-Mortensen, 1985).

ARTERIAL-VENOUS DIFFERENCE.

Theoretically, the renal and total body clearances should be estimated using arterial blood as, clearance is defined as the volume of plasma flowing through the organ of elimination completely cleared of inulin per unit time, and this occurs from the arterial side. In practice, venous blood is used, but this should not cause large errors unless, there is a significant arterio-venous difference in concentrations (Wilkinson, 1987). After an intravenous injection, uptake of the marker from the circulation into tissues, means arterial concentrations are initially high, but during the subsequent post-equilibrium elimination phase, the venous concentrations become greater due to reversal of this movement. The clearance estimated from this venous plasma will be lower than if calculated using arterial plasma (Brun et al, 1949; Chiou & Lam, 1982).

This is not a problem with the constant infusion technique, where steady state plasma concentrations are achieved (Smith, 1951), or with methods based on the plasma concentration-time curve taken from zero time to infinity, as the total area under the arterial and venous plasma concentration-time curves should be identical (Chiou & Lam, 1982).

DELAY TIME

This has already been discussed in the context of the constant infusion method. In single injection techniques, it only becomes a problem if urine is collected, but the errors can be minimised by increasing the duration of the collection periods (Pihl, 1973).

Inulin kinetics after a single injection

Following a rapid intravenous injection of inulin, distribution equilibrium is thought to be reached within 40 to 60 minutes. There is net transport of inulin from the plasma to interstitial fluid up to this time, after which movement occurs in the opposite direction (Robson et al, 1950; Schachter et al, 1950). Brun et al (1949), found that venous concentrations of inulin were on average 7% higher than the simultaneously determined arterial concentrations in 4 subjects, two hours after an intravenous bolus. Ferguson et al (1950), reported an average arterial venous difference for 7 subjects of +1% to -3% throughout the duration of the study over a similar time period, while in another study in infants no difference could be found (Broberger, 1973).

Inulin disposition appears to be consistent with a two compartment model, in which distribution equilibrium is attained between 40 to 60 minutes after the injection. (Alving & Miller, 1940; Denneberg et al, 1961; Ladegaard-Pederson, 1972; Broberger, 1973; Fawer et al, 1979; Rehling et al, 1984). The same seems to apply to Poly-fructosan-S (Falbriard & Zender, 1964; Muller-Suur et al,

1983). However, Robson et al, (1950), observed a smooth curvilinear concentration-time relationship after an intravenous bolus of inulin, but in no subject did the logarithm of the plasma concentration bear a linear relationship to time.

The measurement of inulin clearance as a measure of GFR today

The use of inulin to measure GFR is now limited to research, except possibly in paediatrics. It is also used in conjunction with the constant infusion method, as the reference standard for validating other methods. Its demise is due to two main reasons. 1) The chemical determination of inulin is laborious and non-specific. (The former problem has now been overcome, to a certain extent, by the automation of the technique (Dawborn, 1964). 2) The constant infusion method is tedious and cumbersome and therefore not clinically practicable (Kampmann & Molholm-Hansen, 1981). Inulin is used in research because it is the most reliable marker known for the estimation of GFR, and the constant infusion is a good method for studying dynamic changes over time (Materson, 1971). Inulin has been replaced to some extent by other endogenous and exogenous markers, not because they more accurate than inulin but because their analysis is easier.

ENDOGENOUS AND EXOGENOUS MARKERS

Urea

Urea is unsuitable for measurement of GFR because its clearance depends on the urine flow rate (Schuster & Seldin, 1985).

CREATININE

The endogenous creatinine clearance is measured most extensively as an index of the GFR in clinical practice (Schuster & Seldin, 1985). It is normally estimated from

the creatinine content of a 24 hour urine collection, and one blood sample. The creatinine clearance is influenced by numerous factors including the rate of creatinine synthesis, the method of analysis, and renal function.

Creatinine is produced by the spontaneous, non-enzymatic dehydration of creatine and phosphocreatine. About 2% of the creatine in muscle is converted to creatinine daily (Borsook & Dubnoff, 1947), and patients with decreased muscle mass (e.g. the elderly), have lower rates of creatinine production (Cockcroft & Gault, 1976). Depending on the method of analysis, errors may arise because of interfering chromogens such as acetoacetic acid and acetone in the blood (but not urine) (Kampmann & Molholm-Hansen, 1981). The creatinine clearance determined by autoanalyser methods, is comparable to the inulin clearance, because the plasma creatinine concentrations are overestimated (Healey & Graeme, 1968).

Creatinine is secreted by the renal tubules (Smith, 1951), and the 'true' ratio of the creatinine to inulin clearance ranges between 1.01 to 1.68 (Healey & Graeme, 1968). Measurement of the creatinine clearance also requires accurate urine collections. Although short urine collection periods are convenient, and the midpoint plasma sample is likely to be representative, inaccurately timed collections and incomplete bladder emptying can cause major errors, unless the subject is supervised and hydrated (Doolan et al, 1962; Richardson & Philbin, 1971). With a 24 hour collection period, the clearance is not dramatically influenced by small errors in timing or residual urine, and diurnal variation is taken into account, but a single plasma sample may not be representative (Camara et al, 1951; Pasternack & Kuhlback, 1971). The plasma creatinine concentration depends on physical activity and diet, the latter being increased by eating cooked meat (Mayersohn et al, 1983). The errors associated with diet, activity, diurnal

variation and incomplete 24 hour collections produce a day to day variation of 25% (Zender & Falbriard, 1967; Brochner-Mortensen & Rodbro, 1976a). As renal function declines in patients with renal disease, the creatinine clearance progressively overestimates the GFR, as the proportion secreted increases (Bauer et al, 1982; Shemesh et al, 1985). Despite the many problems, nomograms and formulae have been devised to take muscle mass, age and sex into consideration. These allow the creatinine clearance to be predicted from a single plasma creatinine concentration (Cockcroft & Gault, 1976).

Exogenous markers

The majority of exogenous markers are radiolabelled compounds, which are easy to measure. They include radiolabelled inulin, vitamin B12, chelating agents, contrast media, allantoin, mannitol, sucrose, thiosulphate, polyethylene glycol, dextrans, glucosamine and ferrocyanide (Levinsky & Levy, 1973; Materson, 1971; Bianchi, 1972). Today, the fashionable radiolabelled compounds are iothalamate (I^{125}, I^{131}) and Cr^{51} ethylenediaminetetracetic acid (EDTA).

Iothalamate is not ideal, because it is secreted by the tubules, and it also undergoes extrarenal clearance (Odlind et al, 1985, Prueksaritanont et al, 1986).

Cr^{51} EDTA is the most widely used alternative to inulin. The renal and total body clearance of Cr^{51} EDTA correlates well with the inulin clearance, as measured by the constant infusion method, although, it underestimates it by about 5-15 % (Garnett et al, 1967; Heath et al, 1968; Chantler et al, 1969; Lavender et al, 1969; Hagstam et al, 1974; Kampmann & Molholm-Hansen, 1981). It is mainly used with the single injection technique, using both one and two compartmental model analysis (Truniger et al, 1968; Vogeli et al, 1971; Donath, 1971; Muller-Suur et al, 1983). The latter, is the most accurate method of determination, using the total area under the

curve from zero to infinity (Brochner-Mortensen et al, 1969; Rehling et al, 1984). Simple methods which need only two blood samples have been reported, and although the GFR is overestimated, correction factors may be used (Chantler et al, 1969; Brochner-Mortensen & Rodbro, 1976b).

Problems associated with the use of radiolabelled tracers

The use of radioactivity needs care and appreciation of the associated dangers. The use of radioactivity is deemed an unnecessary risk in children, and repeated use in adults should be avoided. The potential risk of uptake of iodine 125 & 131 into the thyroid, necessitates pre-medication with potassium iodide (Pearson, 1979). Iodine 131 & 125 and chromium 51 have short half lives, 8, 60 and 27.8 days respectively (Materson, 1971) and therefore, their shelf lives are short. If any metabolism or breakdown of the markers did occur, this would not be not be readily detected.

MEASUREMENT OF RENAL BLOOD FLOW

The kidney is well perfused at a rate of about 4 ml/min/gm tissue. This rapid renal blood flow has three major functions :-

1) to supply oxygen and nutrients.
2) to deliver metabolic waste for elimination.
3) to regulate renal function (e.g. glomerular filtration, reabsorption, secretion, and concentration of urine - the important events in urine formation).

90 % of the renal blood flow perfuses the cortex, and 10% the medulla (Brenner et al,1987). The renal blood flow is kept constant over a wide range of blood pressures by autoregulation, which is maintained through intrinsic vascular tone and vasoactive hormones such as, vasopressin and catecholamines (Pearson, 1979; Baylis, 1986). Renal blood flow can be altered by disease states (e.g. renal artery stenosis), physiological conditions (e.g.

pregnancy) and drugs (e.g. non-steroidal anti-inflammatories) (Pitts, 1968; Dunn, 1984).

Renal blood flow can be measured directly by renal vein cannulation, and local thermodilution, but these methods require general anaesthesia, and are not practical for clinical use (Levinsky & Levy, 1973).

Indirect techniques are based on the Fick principle (Brenner et al, 1987), where a quantity of a chemical marker (Q), removed from the plasma by the kidney, is equal to the amount present in the renal artery (Qa) minus the amount present in the renal vein (Qv), multiplied by the renal plasma flow, (RPF). Thus :-

$$Q = RPF \times (Qa - Qv) \qquad \text{(Equation 13)}$$

If the marker is not synthesised, metabolised or reabsorbed by the kidney, the quantity extracted Q, will equal the amount excreted in the urine (Ux V), where Ux is the concentration of x in the urine, and V is the urine flow rate. So, by substituting and rearranging equation 13 :-

$$RPF = \frac{Ux\ V}{(Qa - Qv)} \qquad \text{(Equation 14)}$$

If the marker is 100% extracted in one passage through the kidney, then the amount of marker present in the renal vein (Qv) will equal zero.

So
$$RPF = \frac{Ux\ V}{Qa} \qquad \text{(Equation 15)}$$

The renal blood flow can now be calculated, if the proportion of blood to plasma is known from the haematocrit, (HCT)) so:-

$$RBF = RPF/(1 - HCT) \qquad \text{(Equation 16)}$$

where the haematocrit is expressed as a fraction. In the case of compounds that are not 100 % extracted, the renal blood flow can be calculated from the following equation:

$$RBF = RPF/\ Ex\ (1\text{-}HCT) \qquad \text{(Equation 17)}$$

where Ex is the extraction ratio of the compound being

measured (Schuster & Seldin, 1985).

Historically, the first substance tested was phenol red, but it had only a 70 % extraction (Marshall, 1931). This was followed by hippuran, an iodinated hippuric acid compound used in urography (Elsom et al, 1937). Smith et al, (1938), realised that the plasma clearance of a substance by the kidney must have an upper limit, which is determined by the renal plasma flow, and they reported that the clearance of diodrast could be used as a direct measure of the RBF in man. It was later replaced by p-aminohippuric acid as this was easier to assay (Smith et al, 1945). p-Aminohippuric acid has since become the standard for measuring renal blood flow.

p-Aminohippuric acid

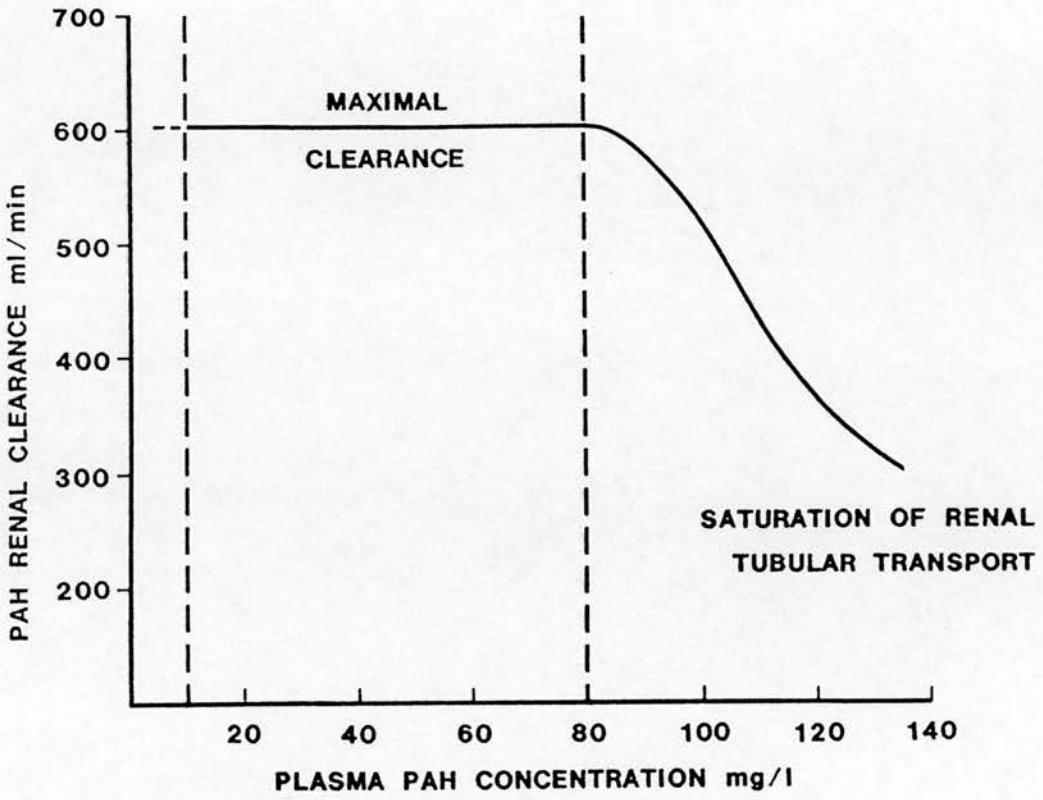
p-Aminohippuric acid (PAH) is an organic anion of molecular weight 194.2, with a pKa of 3.6. PAH is a major metabolite of p-aminobenzoic acid, being formed in the body by its conjugation with glycine. It is freely soluble in water as the sodium salt (Beyer et al, 1945). PAH is partially plasma protein bound (10-20 %, Taggart, 1951), and the free fraction is filtered by the glomerulus. A further fraction is secreted across the proximal tubule by active transport, via a specific organic anion transport system (Pitts, 1968). The secretion and uptake of PAH by the proximal tubule has been widely studied, both "in vivo" and "in vitro" (Moller & Sheikh, 1983; Weiner, 1985). The inhibition of active PAH secretion reduces its clearance to the level of the glomerular filtration rate. Metabolic poisons (e.g 2,4, dinitrophenol), direct competition for the transport system (eg sulphonamides), disease states and saturation of the transport system by PAH itself, all depress the secretion of PAH (Moller & Sheikh, 1983; Weiner, 1985). The saturation of PAH transport means that its clearance is maximal at low plasma concentrations (below 30 mg/l). At plasma concentrations of 400 to 600 mg/l, the tubular

secretory capacity for PAH is saturated (Smith, 1951) (Fig 1.5).

The extraction of PAH is decreased at plasma concentrations above 30 mg/l in patients with impaired renal function (Chasis et al, 1945). Thus, the tubular secretion of PAH is limited by a maximal rate (TmPAH), and this can be measured to estimate tubular excretory function in health and disease (Chasis et al, 1945; Smith, 1951).

FIG 1.5

Relationship of PAH clearance to plasma PAH concentration



The amount of PAH extracted (Epah) in one passage through the kidneys, can be estimated if renal vein catheterization is performed. In health this can vary between 81% and 100% (mean 91 %) (Smith, 1951). Thus, the PAH clearance underestimates the true renal plasma flow, and can only be used as a measure of effective renal plasma flow (ERPF). This can be corrected for if, the extraction ratio is known. The extraction ratio will be

affected by factors which interfere with the secretion of PAH, as detailed above.

The clearance of PAH is only a measure of cortical blood flow. Medullary blood flow is thought to make up 10-20% of the total (Berger and Herd, 1971), and this may explain why PAH is only 90 % extracted. It has been proposed, that the unextracted PAH fraction may be used to calculate the medullary blood flow (Reubi, 1958), but this is an overestimate (Levinsky & Levy, 1973). Any diversion of blood from the renal cortex, will decrease the extraction of PAH.

PAH clearance as a measure of renal plasma flow

The validity of PAH clearance as a measure of RBF depends on the fulfilment of certain criteria (Cohen, 1974) :-

1) Complete elimination from the blood in one passage through the kidney
2) No synthesis, metabolism or storage in the kidney.
3) It must be physiologically inert.

PAH is accepted as the best available compound for measurement of the RBF as, it is almost completely extracted during one passage through the kidney (90%) at low plasma concentration, having a maximal constant clearance with plasma concentrations between 7 and 30 mg/l (Smith, 1951). In subjects with normal renal function, the secretion of PAH is a linear function of its concentration in plasma, until this is raised over 80 mg/l, at which level the secretory mechanism begins to saturate. The maximal rate of PAH secretion (Tmpah) can be estimated where Tm is equal to the maximal rate of net transport, which is in the region of 80 mg/min/1.73m^2 (Pitts, 1968; Schuster & Seldin, 1985). It is not thought to be taken up by erythrocytes (Smith et al, 1945). PAH is thought to be eliminated solely by the kidney, and it is easily analysed in plasma and urine using colorimetry

or high performance liquid chromatography (Smith et al, 1945; Meerdink et al, 1981).

The use of PAH clearance as a reliable estimate of RBF has been questioned, because PAH is metabolised to some extent to the N4 acetyl derivative (Fig 1.6) both in animals and man (Smith, 1951). This metabolic loss is thought to be insignificant, and to occur extrarenally (Smith, 1951; Pearson, 1979). However, there is other evidence that PAH is metabolised in the kidney in animals (Setchell & Blanch, 1961; Gyrd-Hansen & Rasmussen, 1970; Malyusz et al, 1979; Carpenter & Mudge, 1980). In man, kidney acetylation of PAH has been demonstrated "in vitro", (Frindt & Vial, 1968) and may occur "in vivo", (Newman et al, 1949; Grindt et al, 1974). The latter investigators, have also reported that PAH is deconjugated to p-aminobenzoic acid and its acetyl derivative (Fig 1.5).

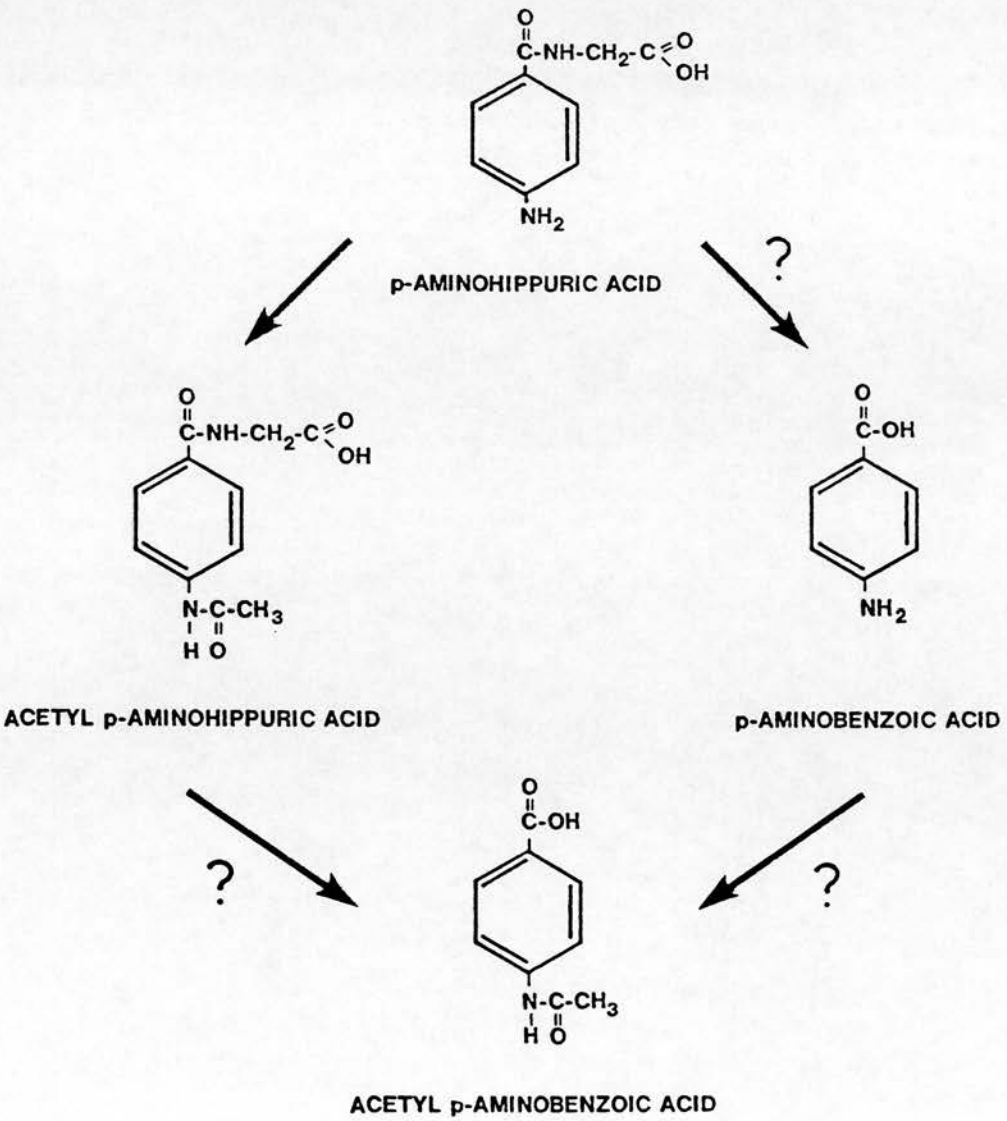
Despite these potential problems, the PAH clearance, determined with the classical constant infusion technique remains the standard reference method for measuring RBF (Pearson, 1979; Brenner et al, 1987).

CLEARANCE TECHNIQUES-p-Aminohippuric acid

The standard method for measuring PAH clearance is the classical infusion method of Smith (1951), as described for inulin (page 14). The steady state plasma concentration of PAH must be kept below 30 mg/l, to insure maximum extraction. The problems with the constant infusion of PAH are the same as with inulin, (eg the need for complete accurately timed urine collections). Other aromatic amines (eg sulphonamides, and procanamide), interfere with the colorimetric analysis of PAH (Waugh & Beall, 1974), and high plasma glucose levels depress the PAH clearance by a direct effect on tubular transport or, formation of a glucose-PAH complex, which may not be detected (Lote et al, 1985; Greene et al, 1987).

Fig 1.6

The structure of p-aminohippuric acid and its potential metabolites.



The average value for PAH clearance (effective renal plasma flow), for the age range 20 to 40 years are 654 ± 163 and 592 ± 153 ml/min/1.73 m^2, for men and women respectively. Thus, the average effective renal plasma flow shows a wide variability, with the 95 % confidence limits for PAH clearance ranging from 328 to 980 in men, and 268 to 898 ml/min/1.73 m^2 in women. If it is assumed that the average Epah is 0.9, then the renal plasma flow will be in the range 364-1088 and 298-998 ml/min/1.73 m^2 respectively. Again, taking an average haematocrit for males as 45% and females 40%, the total renal blood flow will be 662-1978 and 497-1663 ml/min/1.73 m^2 respectively (Smith, 1951; Wesson, 1969). Davies and Shock (1950b), showed that the diodrast clearance (which has a similar clearance to that of PAH), determined in a single person on different days, would have to differ by more than 165 ml/min/1.73 m^2, to represent a significant change at the 5% level. There is also diurnal variation in the PAH clearance as with inulin (Wesson, 1969), and age also influences the PAH clearance. In infants up to three months of age, the Epah is approximately 60%, whilst after 50 years, the PAH clearance falls by 20 ml/min/ decade. (Davies and Shock, 1950a).

The use of PAH clearance as a measure of RBF in patients with renal disease, depends on the extent of extraction of PAH. In glomerulonephritis, and nonspecfic nephropathies, the PAH extraction is reduced in some patients (Brodwell, 1964). Therefore, when using PAH to measure RPF in renal disease, it is necessary to know its extraction ratio (Schuster & Seldin, 1985).

Simplification of the constant infusion method to measure PAH clearance has been attempted, in the same way as for measuring the clearance of inulin.

The constant infusion of PAH without urine collection

The renal plasma flow was first measured using this technique by Earle and Berliner (1946). When the plasma

samples were hydrolysed to determine total PAH (as a small proportion of PAH is metabolised), a good correlation with the standard method was attained (Berger et al, 1948). Without the hydrolysis step, the total body clearance of PAH overestimated the simultaneous calculated renal clearance by 20-30 %. This was thought to represent the amount eliminated by metabolism (Cole et al, 1972, Statius Van Eps, et al 1967). More recently, Schnurr et al (1980), reported a good correlation between the total clearance and renal clearance using this method, but they did not state whether the analytical method included hydrolysis. The disadvantages of this technique have already been presented (page 18, and Table 1.2, p 26). One other potential source of error with this method is, the possibility that products other than PAH will be formed during hydrolysis, and that these will give a positive reaction on analysis (i.e p-aminobenzoic acid, Brown et al, 1976)

The single injection method

Single intravenous injection techniques have been used to estimate PAH clearance both with, and without the collection of urine. Early workers, used single injection with urine collection and calculated the renal clearance from the amount excreted divided by the midpoint plasma concentration, taken from the semilogarithmic plot against time (Landowne and Alving, 1947; Newman et al, 1949). The latter reported that in man (but not in dogs), the clearance of PAH fell, as the plasma concentration declined. Neither group validated their results by comparison with the classical infusion method.

Tacket and Houck (1950), calculated the total body clearance of PAH on the basis of a one compartment pharmacokinetic model, and claimed that a semilogarithmic plot of the plasma decay curve against time was linear, 50 minutes after the injection. With this model, they only obtained a good agreement between the PAH clearance

and the simultaneously calculated renal clearance, if the distribution volume of mannitol was used, instead of that of PAH. They also reported that the average renal clearance of PAH increased, as the plasma concentration declined. Rosenbaum et al, (1973), used a one compartment model, with plasma concentration data 30 to 45 and 45 to 60 minutes after the injection, and found that the plasma clearance of PAH consistently overestimated the values obtained by the standard infusion method. Similar findings were described in children, but the model was not specified (Boineau et al, 1974).

Mandel et al, (1955), used a two compartment model in dogs, and found good agreement in 11 of 15 studies, with the PAH clearance estimated from a constant infusion.

PAH was apparently cleared by patients with no kidneys after a single injection, using a one compartment model (Rosenbaum et al, 1973), but this was not confirmed in anuric patients, with a hydrolysis step during analysis of the plasma (Tacket & Houck, 1950). The clearance of PAH following a single injection is lower if peripheral venous, rather then arterial plasma concentrations are used (Newman et al, 1949; Tacket & Houck, 1950). The venous clearance was 16 % lower than those calculated using arterial clearance (Tacket & Houck, 1950). The distribution of PAH is thought to be complete by 30 to 50 minutes, after an intravenous bolus injection (Tacket & Houck, 1950; Rosenbaum et al, 1973). The potential problems with single injection techniques have been mentioned previously (pages 25-28, and Table 1.2, p 26).

Other methods for the estimation of the PAH clearance by single injection have been proposed, using intramuscular, (Bucht, 1949) and subcutaneous (Brun, 1951) routes. These methods cause pain on administration, and the rate of absorption is variable.

OTHER TEST COMPOUNDS

Radiolabelled substances, including I^{125} and I^{131} hippuran (ortho-iodohippurate) and I^{131} diodrast (iodopyracet), have been introduced as alternatives for PAH. Their clearances, after a single injection, correlate well with the simultaneously determined PAH clearance, during a constant infusion (Elwood et al, 1965; Maher et al, 1971; Silkalns et al, 1973; Pearson, 1979). Predictably, two compartmental pharmacokinetic analysis is the most accurate model for determination of I^{131} hippuran clearance. Simplification, with reduction in the number of blood samples and the use of a one compartment model, overestimates the true clearance (Taylor et al, 1985). The major disadvantage of the use of radiolabelled iodinated compounds is, the risk of uptake into the thyroid gland (Pearson, 1979). The clearance of hippuran after single injection has a coefficient of variation of 25% (Favre, 1978). The renal clearance of radiolabelled iodohippurate has been shown to decline with time, and falling plasma concentrations (Pihl, 1973), and radiolabelled iodopyracet clearance is reduced at low plasma concentrations, this being attributed to increased plasma protein binding (Block & Burrows, 1960).

Radioactive isotopes such as 131 Xenon have been used for measuring renal blood flow, by the gas washout technique (Pearson, 1979).

SUMMARY

Glomerular filtration is a major mechanism of renal excretion, and the methods used for its measurement are based on the clearance of a specific marker. The universal standard is inulin, but measurement of its clearance by the constant infusion method is time consuming, and subject to errors, because of inaccurate urine collections. Single injection techniques are more

practicable for routine use, but proper pharmacokinetic analysis using either, open two compartment model or model independent methods, are essential. The endogenous creatinine clearance is the most widely used index of GFR. It is convenient, and analysis are routine in most hospitals, but it is far from ideal. Radiolabelled markers have been used in some establishments, for the routine measurement of GFR. Cr^{51} EDTA is a popular replacement for inulin, but it underestimates the inulin clearance by 5-15 %. Radiolabelled markers and creatinine are only used because of ease of analysis.

Because of the problems associated with radioactivity, and the unreliability of the measurement of creatinine clearance, the single injection of inulin has been re-evaluated as a simple, safe, practicable and reproducible method of estimating the GFR.

Measurement of the renal clearance of PAH by the constant infusion method, is thought to be a good estimate of the renal plasma flow, and has long been the standard reference method. Due to its limitations, this method is mainly restricted to research applications. Radiolabelled I^{131} hippuran and diodrast have been introduced as clinically practicable substitutes for PAH. Their clearances can be estimated using single injection methods, and appropriate pharmacokinetic analysis. Measurements of the PAH clearance have been attempted by the constant infusion method without urine collection, and by single injection. These methods estimate the total body clearance of PAH, and this is an overestimate of the renal clearance because there is significant extrarenal elimination of PAH by acetylation. Thus, methods in which urine PAH is not determined, will be inaccurate. There is evidence that PAH is metabolised by the kidney, and this raises the possibility that the measured renal clearance of PAH, actually underestimates its true clearance.

The disposition and formation of acetyl PAH and its

influence on the measurement of PAH clearance, has been investigated following a single injection and during constant infusion of PAH.

CHAPTER TWO

SINGLE INJECTION METHOD FOR THE MEASUREMENT OF GLOMERULAR FILTRATION RATE USING INULIN

SECTION I

Evaluation of a single injection technique for the measurement of inulin clearance

INTRODUCTION

Accurate measurement of the glomerular filtration rate (GFR) is necessary to assess changes induced by drugs, disease or physiological factors. Measurement of the renal clearance of inulin during constant intravenous infusion at steady state plasma concentrations, is the reference technique. However, this technique is tedious and cumbersome for both patient and investigator, and it requires short, accurately timed complete urine collections. This necessitates bladder catheterization, which is unpleasant, and carries the risk of urinary tract infection. In addition, the obligatory water diuresis places the kidneys in an abnormal physiological state. These factors, combined with lengthy immobilisation of the patient, make this method impractical for routine clinical use, and it is now reserved mainly for research purposes (Kampmann & Molholm-Hansen, 1981). The creatinine clearance is used as the standard clinical indicator of GFR, however, it overestimates the true GFR because of tubular secretion, and this disparity increases with decreasing GFR (Bauer et al, 1982). More convenient and accurate clinical assessments of GFR are now being calculated from plasma concentration-time decay curves, following single injection of radiolabelled tracers (Brochner-Mortensen, 1985). The two most commonly used are Cr^{51} EDTA and I^{131} iothalamate. Cr^{51} EDTA underestimates the inulin clearance by 5-15%, and the suitability of iothalamate has recently been questioned, because of evidence of tubular secretion and extrarenal elimination (Brochner-Mortensen, 1985; Odlind et al, 1985). These techniques have become widely accepted for clinical use, as urine collections are not required.

The measurement of the inulin clearance following a single intravenous injection has been limited, even though it is the standard marker for the estimation of GFR. The early methods still required urine collections,

and were based on inappropriate pharmacokinetic analysis; some workers reported a fall in the inulin clearance following a single injection (Josephson & Lindahl, 1943; Barnard et al, 1955). More recently, the single injection of inulin has been reported in children (Broberger, 1973; Muller-Suur et al, 1983), but studies in adults are limited, and results have not been compared with the reference method (Ladegaard-Pedersen, 1972; Rehling et al, 1984). With the increasing recognition of pharmacokinetic principles and their correct application for the estimation of clearance, the use of single injection techniques is practical for the clinical measurement of the GFR. As inulin is still the reference standard, and the problems associated with analysis have been partially overcome with the introduction of automated methods (Dawborn, 1964), it seems that the inulin single injection technique using appropriate sampling and kinetic analysis, would be preferred for the estimation of GFR in adults. The technique is simple, safe and practical, and it obviates the need for accurate urine collections, and the use of radioactive markers. This is particularly important in studies with repeated measurements.

METHODS

The inulin clearance was measured in 10 healthy male volunteers, aged between 21-50 years (mean 26 $\pm$ 9 years) weighing 74 $\pm$ 7 Kg (range 63-83 Kg) by both the traditional constant infusion method, and a single injection technique. The methods were used in random order, and the two studies were carried out within fourteen days.

Volunteers

The volunteers were medical students, department staff and healthy males from Edinburgh, recruited by newspaper advertisement. All underwent a physical examination, and were screened for normal biochemical and

haematological values. They gave informed consent before participation, and the study was approved by the local ethical committee.

Procedure

The volunteers fasted from 22.00 hr, and reported to the department at approximately 8.30 am the next morning. The fluid intake for both techniques was similar. The volunteers drank 500 ml of water an hour before the study, and thereafter they drank 100 ml (constant infusion), or 130 ml (single injection) of water every half hour, for 3-4 hours during the constant infusion, and up to 8 hours on single injection days, to ensure an adequate urine flow. On the "single injection" day, subjects were allowed a light lunch at four hours. The subjects remained supine for the first four hours on single injection days, and for the duration (3-4 h) of the constant infusion. The same batch of inulin (Kerfoot, Barnsley. U.K.), was used for each individual, on both study days. It was prepared by heating a 50 ml (10 % w/v, 5g) ampoule in a beaker of water at 80°C for 15 minutes, until a clear supersaturated solution was obtained. The solution was cooled before administration. p-Amino-hippuric acid was also administered in the following studies, and the results appear in chapter 3.

Constant infusion method

The inulin clearance was measured by the traditional constant infusion method of Smith (1951), as modified by Mackay et al, (1984). On arrival at the unit, the volunteers emptied their bladder, and an aliquot of urine was kept as a control blank. The subject was weighed, and cannulae (Venflon, 1.2 mm O.D), were inserted into veins in each forearm. A control blank blood sample (10 ml), was drawn. One cannula was attached to an infusion pump (Braun Perfuser) via an infusion line, and a loading dose of 2.3 g of 10 % inulin solution was administered over 5

minutes. The infusion was then commenced with a solution containing inulin (18.1 mg/ml, 10 % solution) in 5 % dextrose, given at a rate of 1 ml/min. After an equilibrium period of one hour, the bladder was emptied, and four, half hourly timed urine samples were collected. Venous blood (10 ml) was withdrawn at the start, and end of each urine collection period, using the cannula not used for the infusion. It was kept open by flushing with 1 ml of heparinised saline (10 units/ml), after taking each blood sample.

Single injection technique

The initial procedure for this technique was as described above. Inulin (70 mg/Kg, 50 ml, 10% w/v solution), was given as an intravenous bolus injection. The inulin was injected (Braun Perfuser) over five minutes, after which the infusion line was flushed through with normal saline. The cannula was then removed. Blood samples were collected (10 ml) at 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 180, 240, 360 and 480 minutes after the start of the injection. Urine was collected hourly for the first four hours then at 6, 8 and 24 hours (initially a 4-8 h sample was collected). The inulin syringe was weighed full before and again empty after administration, to allow calculation of the exact dose.

Sample preparation and storage

The blood samples were collected in 10 ml lithium heparin tubes, and centrifuged at 1500 g for 10 minutes. The plasma was removed, and stored in 5 ml plastic tubes at 4°C. Urine volume and pH (Radiometer) were measured, and a 20 ml aliquot was stored at 4°C. The plasma and urine samples were assayed for creatinine and inulin, within 72 hours.

Analytical methods

1) Inulin

Plasma and urine inulin were determined by the method of Heyrovsky (1956), adapted for automated analysis (Dawborn, 1964).

Inulin is hydrolysed to fructose with hydrochloric acid, and on incubation with 3-indolylacetic acid, a purple colour develops.

STANDARDS

Aqueous standards were made by diluting inulin (5g, 10 % w/v solution, Kerfoot. Barnsley, U.K.), or "Inutest" (5g, 25 % w/v solution, Lavevosan-Gesellschaft, Linz, Austria), with distilled water to give solutions containing 50-500 mg/l.

PROCEDURE

Plasma and urine samples were run under the same conditions, using a Technicon Autoanalyser. Plasma samples were estimated directly, and urine was diluted 1 in 10 with distilled water.

The plasma, urine and aqueous standards were diluted 1:2 with 0.5 N hydrochloric acid (BDH, Poole, U.K.), and the inulin was hydrolysed by heating at 60°C for 10 minutes in a double-coil heating bath. The fructose was separated from plasma proteins by dialysis across a Cuprophane membrane (Altex, U.K.), for 7 minutes at 37°C. The protein-free dialysate was then incubated with concentrated hydrochloric acid and 3-indolylacetic acid (BDH, Poole, U.K.) at 60°C for 8 minutes in the second coil of the heating bath, and the colour intensity estimated photometrically at 520 nm. The concentrations of unknown samples were obtained by reference to a standard calibration graph.

2) Creatinine

Creatinine was determined using a standard

autoanalyser method (Gemstar Electro-Nucleonics, W. Germany). The assay is based on the Jaffe reaction, in which creatinine forms a yellow-orange compound with picric acid in alkaline solution. The absorbance change measured at 500 nm, is proportional to the concentration of creatinine, after correction for the reagent blank.

Calculation of clearance

Constant infusion method

Inulin and creatinine clearances were calculated for each half hour collection period using the formula:-

$$Clx = \frac{Ux\ V}{Px} \qquad \text{(Equation 3)}$$

where Clx is the renal clearance of inulin or creatinine, Ux and Px are the urinary and midpoint plasma concentrations respectively, and V is the urine flow rate. The midpoint plasma concentration was taken as the mean of the concentrations at the beginning, and end of each collection period. The clearances for each half hour period were averaged, to give a mean value.

The coefficient of variation for the clearances determined in each collection period, were calculated for each individual by dividing the standard deviation by the mean and multiplying by 100.

Single injection technique

The inulin clearance was calculated from the plasma concentration-time data alone (total body clearance, TBC), and also from the plasma concentration and urinary excretion rate (renal clearance). The area under the plasma concentration time curve (AUC), was calculated by the trapezoidal method.

The TBC was calculated from the following relationship:-

$$TBC = \frac{DOSE}{AUC\ 0\text{-}\infty} \qquad \text{(Equation 8)}$$

The administered dose was estimated by weighing the syringe full prior to, and empty after administration. The weight of 1 ml of inulin was measured for each different batch used, and the dose (g) was calculated from the following equation :-

$$\text{DOSE} = \frac{\text{Weight full - Weight empty}}{\text{Weight 1 ml of inulin}}$$

Zero time for the calculation of the AUC was taken as 2.5 min after the start of the injection, i.e. the midpoint of the period of administration. The time points for calculation were therefore 7.5, 12.5, 17.5, 27.5, 37.5, 47.5, 57.5, 72.5, 87.5, 117.5, 177.5, 237.5, 357.5 and 477.5 minutes.

The total area under the plasma concentration time curve was calculated by pharmacokinetic analysis, based on an open 2 compartment model. A non-linear weighted least squares algorithm (damping Gauss-Newton), was used to obtain the parameters for the best fit of the experimental data, using the microcomputer programme of Yamaoka et al, 1981. From the best fit parameters, the plasma concentration at zero time (Co), and the terminal elimination rate constant, were obtained. The total AUC was obtained by adding the actual AUC (i.e 7.5-477.5 minutes) to the AUC calculated from Co to 7.5 minutes, and the area extrapolated from 477.5 minutes to infinity. This latter area, was calculated by dividing the plasma concentration at the last data point, by the terminal elimination rate constant (Notari, 1987).

The renal clearance (Rcl) of inulin, was calculated using the following relationship:-

$$\text{Rcl} = \frac{\text{amount excreted in urine t1-t2}}{\text{corresponding AUC t1-t2}} \quad \text{(Equation 4)}$$

where t1-t2 is the time of the collection period. The actual AUC was calculated using the trapezoidal rule. The

AUC from zero time (Co) to the first time point, was calculated by back extrapolation to the y axis. The renal clearance was calculated for each urine collection period, except 8-24 hours, and the data for the periods 4-6 and 6-8 hours were combined, to give results for 4-8 hours.

Creatinine clearance was calculated for the same collection periods using equation 3, except 0-1 hours. The creatinine clearance was not calculated for the 0-1 hour collection period, as in the majority of individuals, the timing of this collection period was not always accurate. This did not affect the renal clearance of inulin as, the time from the injection to the end of the first collection period was known, and the relationship of clearance to urinary excretion rate and AUC, is described by equation 4.

All clearances were corrected for a body surface area (SA) of 1.73 m^2:-

$$\text{Corrected clearance} = \frac{Cl \times 1.73m^2}{SA} = ml/min/1.73\ m^2$$

The surface area was estimated from body weight and height by a nomogram (Du Bois & Du Bois, 1916).

Statistical methods

Statistical analysis was carried out using 2 way analysis of variance (ANOVA) to test for differences in clearances between collection periods, against time, and plasma concentration. The significance between means was also determined using a students two tailed "t" test. The Wilcoxon nonparametric rank sum test, was used to determine the statistical significance of differences in the coefficients of variation. The null hypothesis was rejected when $p < 0.05$. Correlation coefficients were calculated by linear regression analysis.

RESULTS

Constant infusion

Plasma inulin concentrations

Steady state inulin plasma concentrations were achieved in each subject. The mean plasma concentrations after the equilibration period to the end of the study ranged from 172 ± 21 to 169 ± 14 mg/l (Fig 2.1.1). Individual plasma concentrations are listed in Appendix I.

Renal clearance of inulin and creatinine

The individual clearances for inulin and creatinine are given in Table 2.1.1 & 2.1.2 respectively. The mean renal clearances of inulin during the four collection periods ranged from 89 ± 14 to 87 ± 11 ml/min/1.73 m^2, and the corresponding creatinine clearances ranged from 120 ± 9 to 106 ± 9 ml/min/1.73 m^2 (Fig 2.1.1). There were no significant differences in clearance between the collection periods for inulin but, there was a significant fall in the creatinine clearance between 1-1½ hours and 2½-3 hours (120 vs 106 ml/min/1.73 m^2, $p < 0.002$)

The mean period to period coefficient of variation in creatinine clearance was 7.7 %, and this was significantly greater than the value of 4.6 % for inulin ($p<0.02$).

Single injection

Plasma inulin concentrations

Following intravenous bolus administration, the plasma concentrations of inulin fell rapidly in all subjects in a curvilinear manner, when plotted semi-logarithmically against time (Fig 2.1.2). The plasma concentration-time curve up to eight hours could be fitted to a three compartment model, and was log-linear only from 4 to 8 hours. Up to two hours it was possible

Fig 2.1.1

Mean plasma inulin concentrations (top) and inulin and creatinine renal clearances (bottom), during a constant infusion of inulin in 10 healthy male subjects. Bars = Standard Deviation.

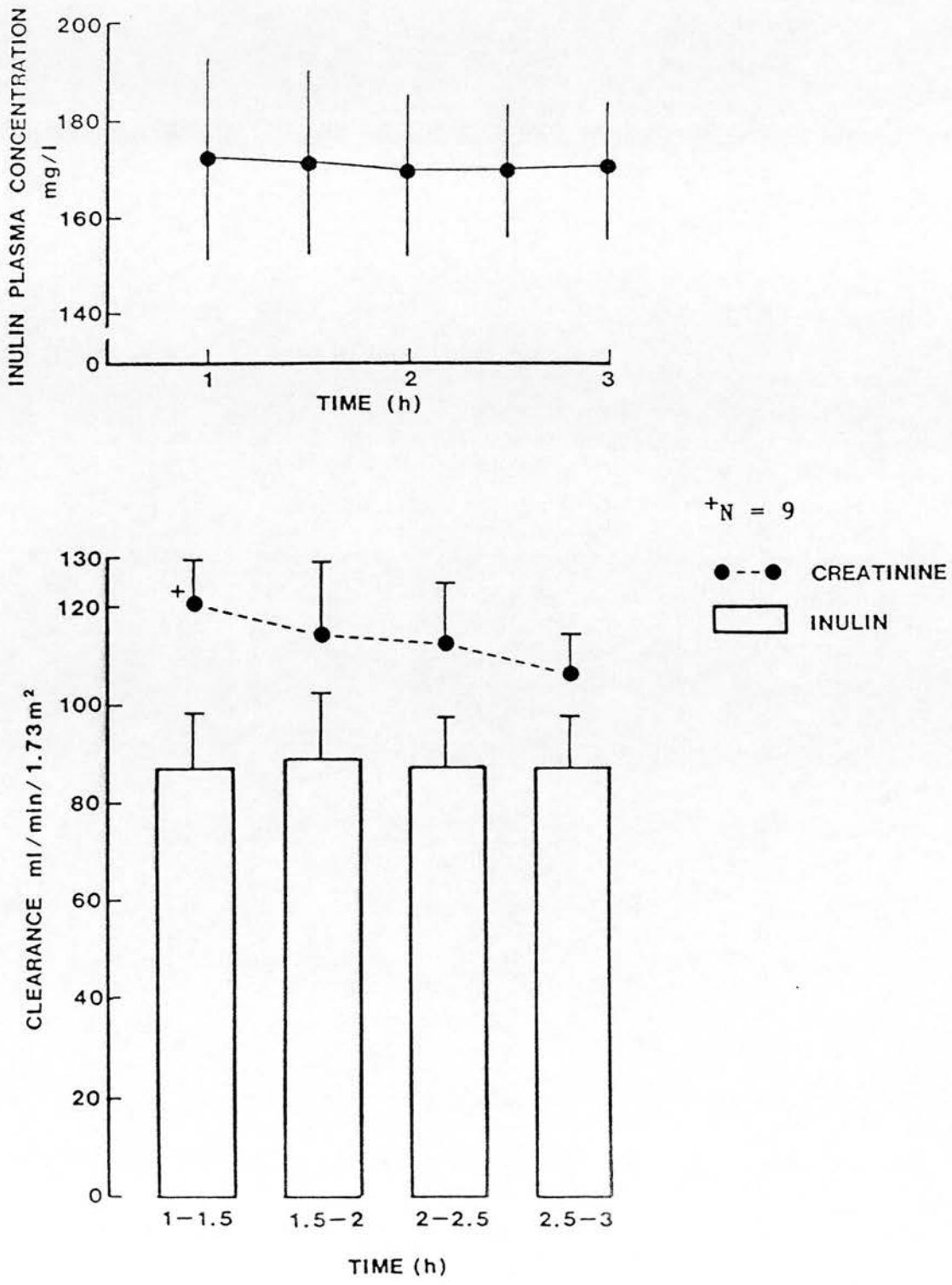


TABLE 2.1.1

The renal clearance of inulin during constant infusion of inulin in 10 healthy males. The clearances are expressed as ml/min/1.73 m^2. The mean ±SD for each subject and each collection period are given together, with the individual period to period coefficient of variation (CV).

	COLLECTION PERIOD (hours)						
SUBJECT	1-1½	1½-2	2-2½	2½-3	MEAN	±SD	CV %
RM	84	81	84	86	84	2.1	2.5
LP	91	88	87	90	89	1.8	2.1
GC	84	85	79	80	82	2.9	3.6
JN	74	70	73	71	72	1.8	2.5
DM	97	92	100	98	97	3.4	3.5
MK	141*	114	92	97	101	11.5	11.4
CP	84	80	78	85	82	3.3	4.0
BH	72	85	88	83	82	7.0	8.5
BS	86	80	83	76	81	4.3	5.3
PL	109	110	107	103	107	3.1	2.9
MEAN	87	89	87	87	88	4.1	4.6
±SD	11	14	10	10	11	3.0	3.0

* Not included in mean due to incomplete bladder emptying at the end of the equilibration period

TABLE 2.1.2

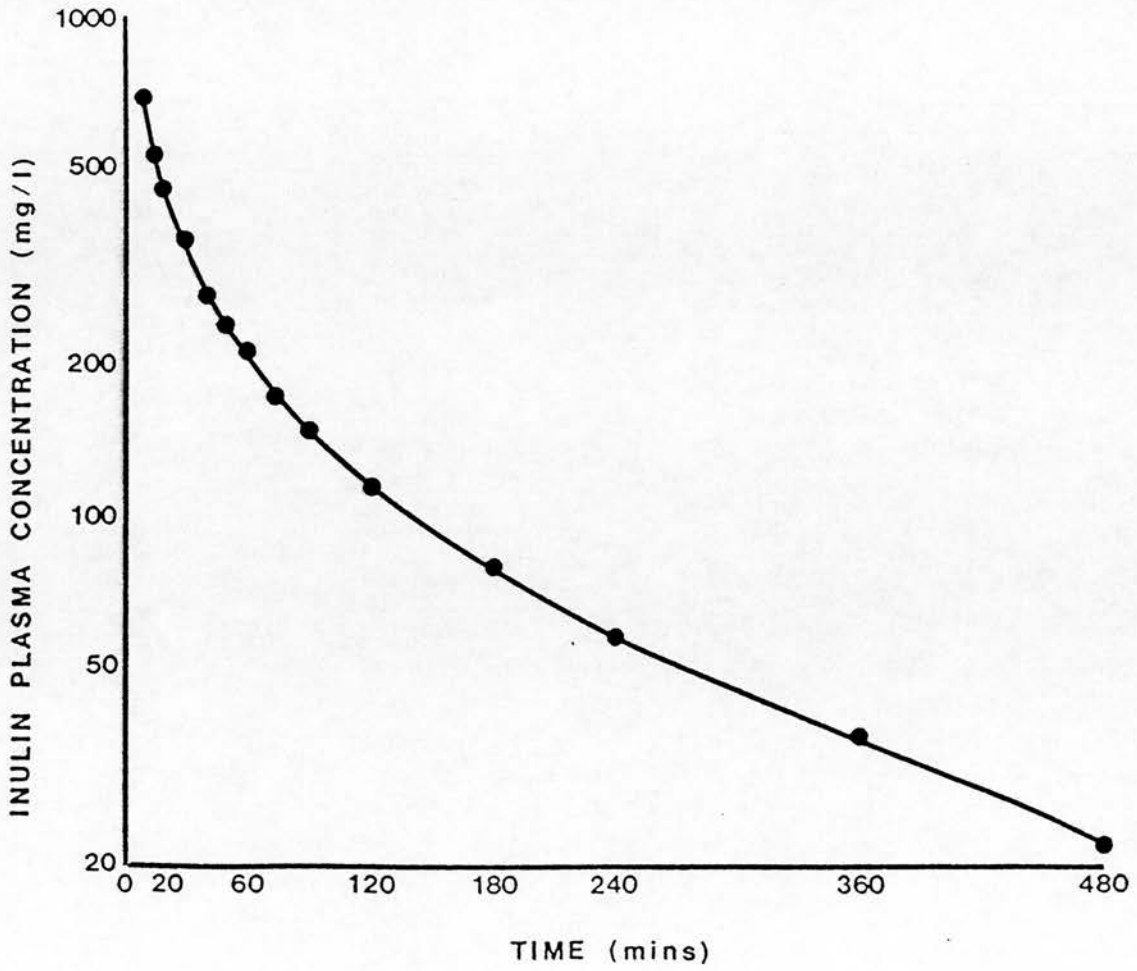
The renal clearance of creatinine during constant infusion of inulin in 10 healthy males. The clearances are expressed as ml/min/1.73 m^2. The mean ±SD for each subject and each collection period are given together, with the individual period to period coefficient of variation (CV).

	COLLECTION PERIOD (hours)						
SUBJECT	1-1½	1½-2	2-2½	2½-3	MEAN	±SD	CV %
RM	136	123	128	124	128	5.9	4.6
LP	108	95	100	98	100	5.6	5.5
GC	124	115	109	107	114	7.6	6.7
JN	117	96	98	94	101	10.6	10.5
DM	124	111	119	108	115	7.3	6.3
MK	163*	119	99	102	107	10.8	10.1
CP	112	105	104	103	106	4.1	3.9
BH	113	142	122	113	122	13.7	11.2
BS	124	101	109	102	109	10.6	9.7
PL	125	131	132	110	124	10.2	8.2
MEAN	120	114	112	106	113	8.6	7.7
±SD	9	15	12	9	10	3.0	3.0

* Not included in mean due to incomplete bladder emptying at the end of the equilibration period

Fig 2.1.2

Mean inulin plasma concentration following a single injection (70 mg/Kg) in 10 healthy male subjects.



to fit the data to a two compartment model. The mean plasma inulin concentrations declined from 686 ± 95 mg/l at 10 minutes, to 22 ± 11 mg/l at 480 minutes. Individual plasma concentrations are listed in Appendix I.

Renal clearance of inulin

The individual renal clearances of inulin for each collection period are given in Table 2.1.3. The mean renal clearance of inulin was relatively constant over the first two hours, but after this time there was a progressive and significant fall ($p < 0.01$). The mean renal clearances were 92, 94, 78, 69 and 48 ml/min/1.73 m^2 over the periods 0-1, 1-2, 2-3, 3-4, and 4-8 hours respectively. The 0-1 hour renal clearance of inulin was significantly greater than at all times after 2 hours ($P < 0.003$) (Fig 2.1.3).

Total body clearance of inulin

The total body clearance of inulin (determined by pharmacokinetic analysis of the plasma concentration-time curve only), also showed a significant fall over time ($p<0.01$). The total clearance determined from time points after 2 hours, was significantly less ($p < 0.02$) than the total clearance from 0-2 hours. The total body clearance fell progressively up to 6 hours, and the mean clearances were 101, 87, 82, 71 and 77 ml/min/1.73 m^2 from 0-2, 0-3, 0-4, 0-6 and 0-8 hours respectively. Individual total body clearances of inulin are given in Table 2.1.4.

Creatinine clearances

The mean creatinine clearances did not differ significantly, and were 117, 113, 110 and 115 ml/min/1.73 m^2 for 1-2, 2-3, 3-4, and 4-8 hours respectively (Fig 2.1.3). The individual creatinine clearances are given in Table 2.1.5. The overall mean creatinine clearance was 114 ml/min/1.73 m^2.

The mean creatinine clearance during the constant

TABLE 2.1.3

The renal clearance of inulin (ml/min/1.73 m^2) at different time periods following a single intravenous injection of inulin in 10 healthy male subjects.

	COLLECTION PERIOD (hours)					
SUBJECT	0-1	1-2	2-3	3-4	4-8	0-2*
RM	87	108	76	78	43	92
LP	85	94	83	67	44	87
GS	87	76	62	48	44	83
JN	83	83	67	70	65	83
DM	108	104	74	56	39	106
MK	91	99	98	63	42	93
CP	90	90	69	73	58	90
BH	73	82	68	65	34	75
BS	96	93	76	74	44	95
PL	121	108	103	95	64	118
MEAN	92	94	78	69	48	92
±SD	14	11	13	13	11	12

* 0-2 hour renal clearance calculated from the 0-2 hour urinary excretion of inulin divided by the area under the 0-2 hour plasma concentration time curve.

Fig 2.1.3

Mean inulin and creatinine renal clearances following a single intravenous bolus of inulin in 10 healthy male subjects. Bars = SD.

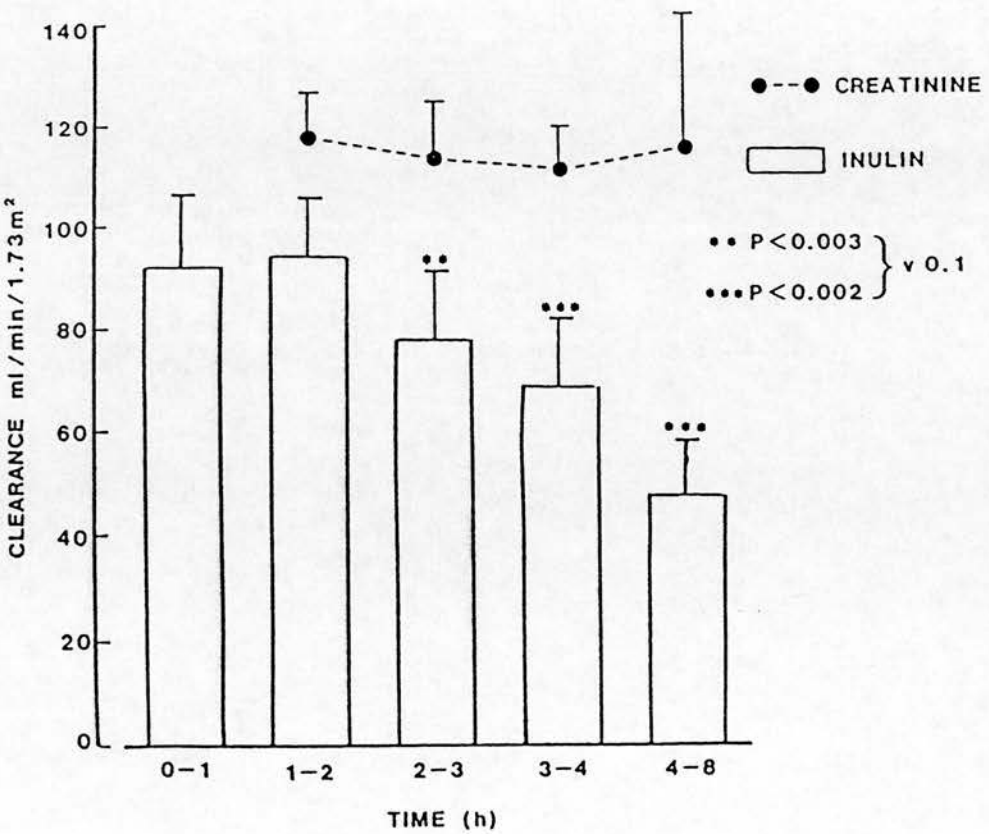


TABLE 2.1.4

Total body clearance of inulin (ml/min/1.73 m^2) at different time periods, following a single intravenous injection of inulin in 10 healthy male subjects

	TIME (hours)				
SUBJECT	0-2	0-3	0-4	0-6	0-8
RM	98	71	81	71	74
LP	91	92	83	53	77
GS	107	91	78	82	82
JN	98	88	87	75	90
DM	104	59	62	76	78
MK	108	108	95	99	96
CP	94	86	79	73	79
BH	92	77	80	38	39
BS	101	90	92	45	67
PL	114	108	85	93	93
MEAN	101	87	82	71	77
±SD	8	15	9	20	16

TABLE 2.1.5

The renal clearance of creatinine (ml/min/1.73 m^2) after a single injection of inulin in 10 healthy males. The mean ± SD for each subject and each collection period are given.

	COLLECTION PERIOD (hour)						
SUBJECT	0-1	1-2	2-3	3-4	4-8	MEAN	±SD
RM	NM	114	110	109	107	110	2.9
LP	NM	97	105	99	105	102	4.1
GS	NM	116	106	113	108	111	4.6
JN	NM	118	96	110	113	109	9.4
DM	129	124	130	126	123	126	3.1
MK	NM	125	127	102	87	110	19.2
CP	131	109	111	99	104	106	5.4
BH	146	119	103	108	105	109	7.1
BS	136	129	115	115	184	136	32.8
PL	151	123	125	123	115	122	4.4
MEAN	117	113	110	115	114	9.3	7.9
±SD	9	11	9	26	10	10	7

NM = Not measured

infusion study, was virtually identical to that determined on the single injection study day (113 ± 10 and 114 ± 10 ml/min/1.73 m^2 respectively).

Comparison of inulin clearance measured by constant infusion and single intravenous bolus injection

The mean 0-2 hour renal clearance of inulin measured by the single injection method, was similar to that determined during constant infusion (92 ± 12 versus 88 ± 11 ml/min/1.73 m^2). The mean ratio of single injection to constant infusion renal clearance was 1.05 ± 0.09, this 5% overestimate is not significant. The clearances determined by the two methods were significantly correlated ($r= 0.77$, $p< 0.01$, Fig 2.1.4). However, the clearance of inulin by constant infusion was significantly greater than the inulin clearance by single injection, during the 2-3, 3-4 and 4-8 hour time periods ($p< 0.01$, Fig 2.1.5). The absolute differences were 10, 19, and 40 ml/min/1.73 m^2

The total body clearance of inulin calculated from the 0-2 hour plasma concentration data after single injection, significantly overestimated the corresponding 0-2 hour renal clearance by single injection (9%, $p< 0.02$), and renal clearance by constant infusion (15 %, $p< 0.002$). There were significant correlations between the 0-2 hour total body clearance and the single injection and constant infusion renal clearances ($r= 0.69$, $p<0.05$ and $r= 0.65$, $p< 0.05$ respectively) (Fig 2.1.6 & 2.1.7). No significant correlation was found between the 0-3, 0-4, 0-6 and 0-8 total body clearances and the constant infusion clearance of inulin.

Fig 2.1.4

Relationship between the renal clearances of inulin determined by the constant infusion and single injection methods, in 10 healthy male subjects.

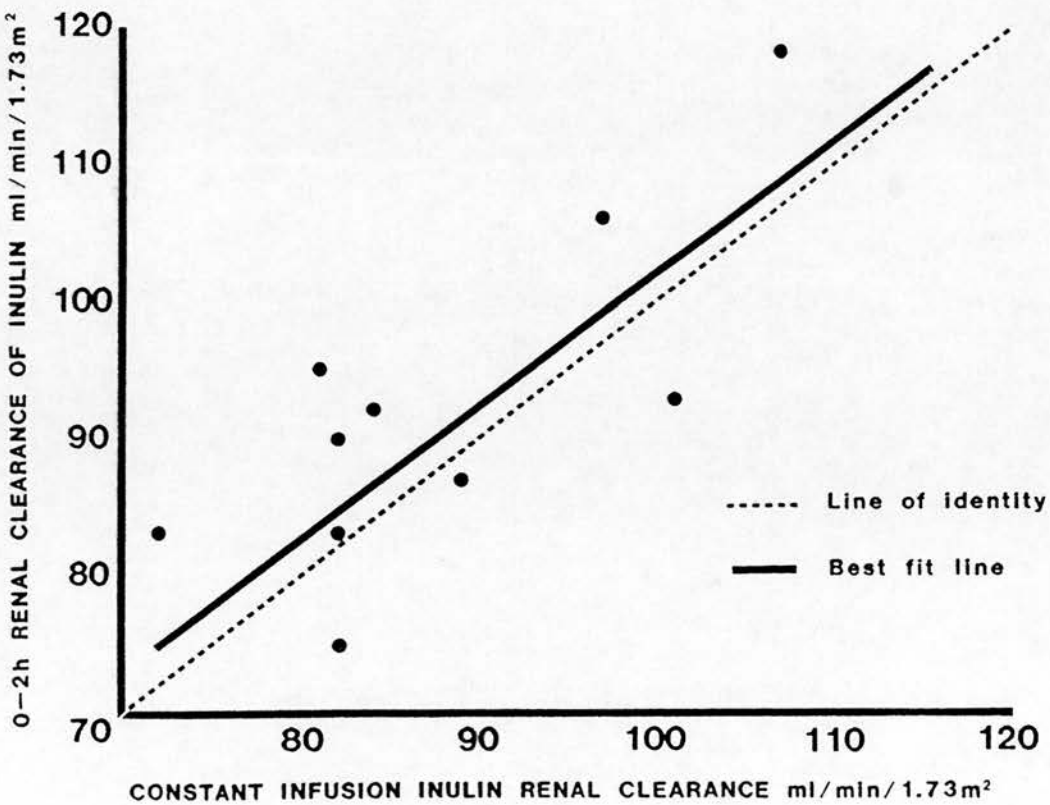


Fig 2.1.5

Mean inulin clearances during constant infusion, and at different times after a single injection of inulin in 10 healthy male subjects. Bars = SD.

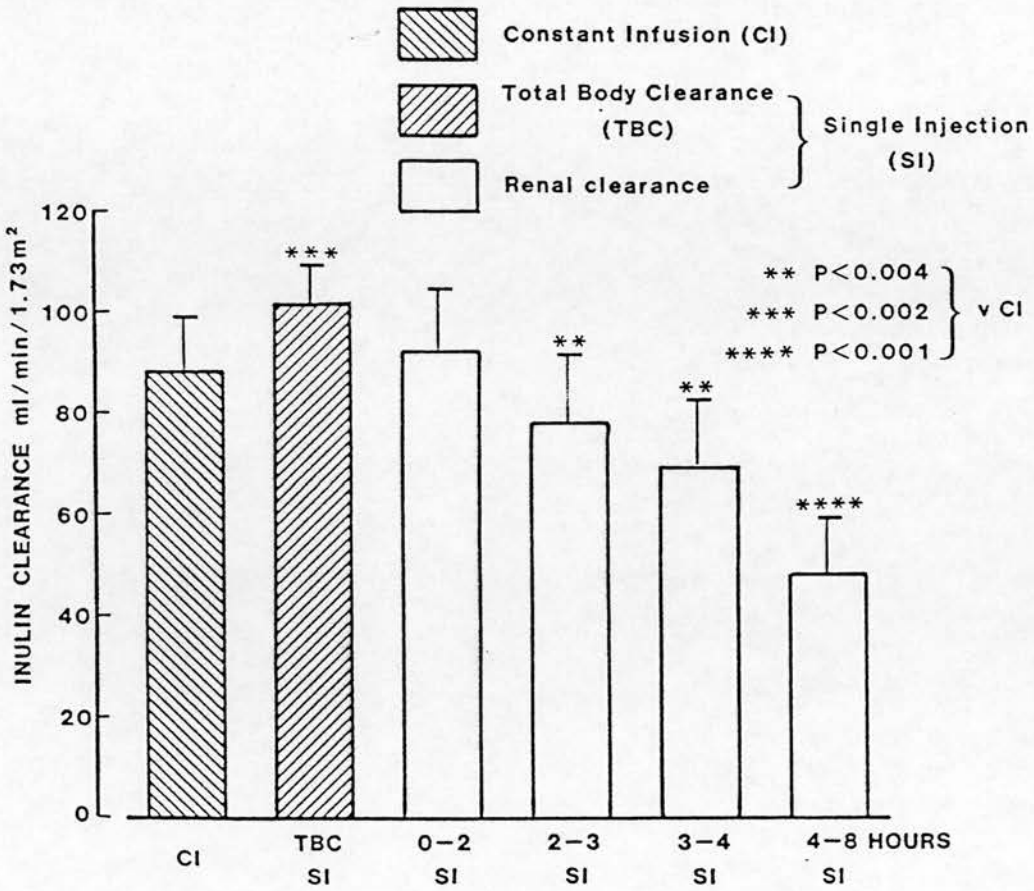


Fig 2.1.6

Relationship between total body and renal clearances of inulin, following single intravenous injection in 10 healthy male subjects.

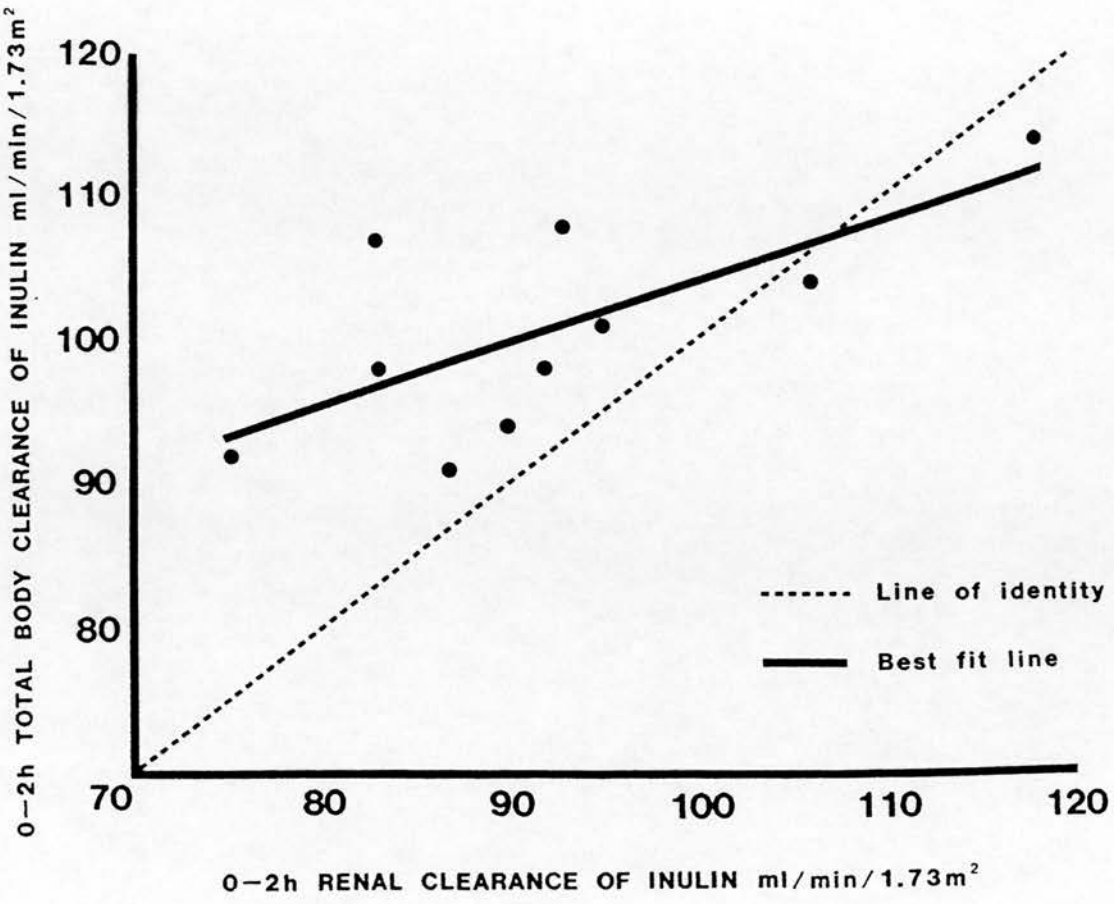
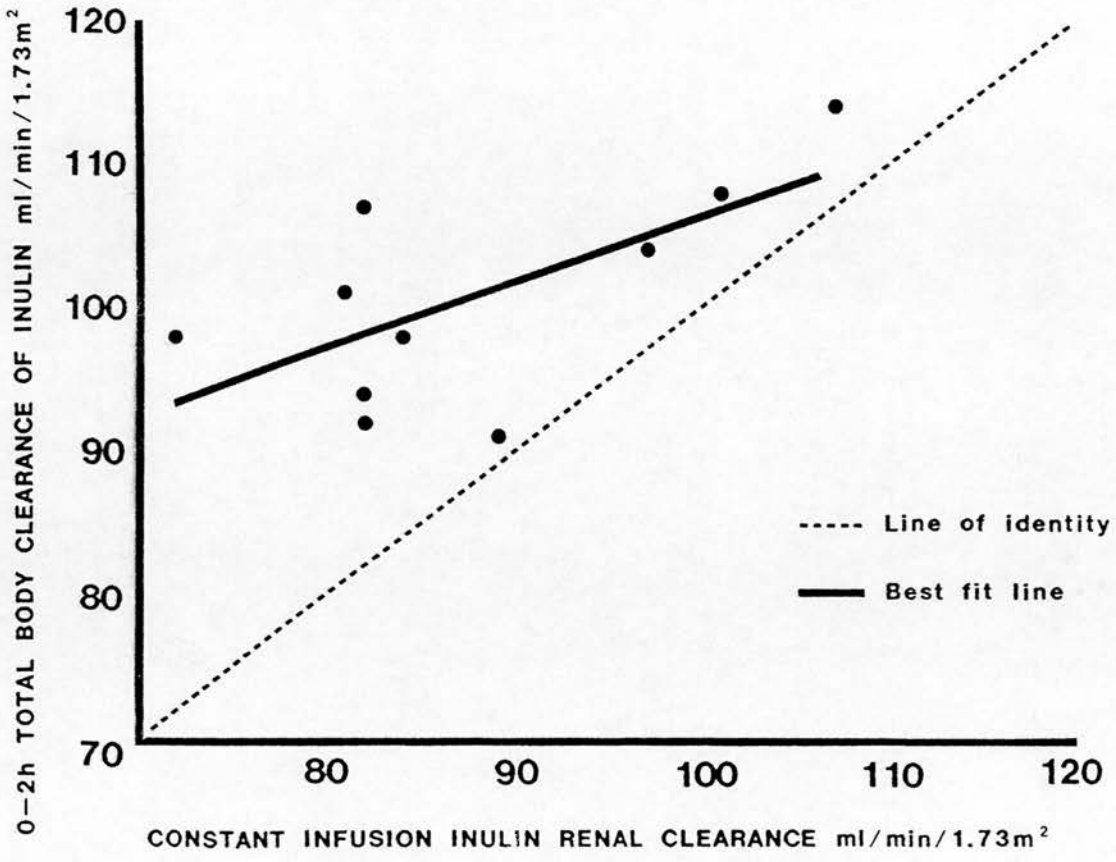


Fig 2.1.7

Relationship between the total body and renal clearances of inulin determined by single injection, and constant infusion methods respectively in 10 healthy male subjects.



DISCUSSION

The renal clearance of inulin measured by constant infusion was consistent over the different periods. However, the average clearances were low compared to the currently accepted average values of 127-130 ml/min/1.73 m^2. The inulin clearances for each individual was however, within the normal range (72-176 ml/min/1.73 m^2, Smith, 1951). The corresponding creatinine clearances were greater than the constant infusion inulin renal clearance, and the mean ratio was 1.28. This difference may be explained by the partial tubular secretion of creatinine (Bauer et al, 1982). The calculated variation in creatinine clearance was greater than the corresponding variation in inulin clearance, over the different time periods. The creatinine clearance thus appears to be a more variable index of the GFR, than the inulin clearance. The mean coefficient of variation between collection periods for the inulin clearance during the constant infusion was 5%, and this is less than previously reported (Smith et al, 1938; Kennedy & Kleh, 1953). This variation could be due to a number of factors including analytical errors, incomplete urine collection and physiological changes in inulin excretion rate (this is discussed in more detail in section II). The collection of urine is the most important source of variation with the constant infusion technique (Zender et al, 1968).

The inulin clearance determined by the single injection method compared well with that obtained by constant infusion, but only over the first two hours. The mean 0-2 hour renal clearance of inulin after single injection, did not differ significantly from that during constant infusion, but was 5% higher. This could possibly be attributed to the fact that the two studies did not occur on the same day. Davies and Shock (1950b), report that the day to day variation in inulin clearance was

greater than the period to period variation. In the only other reported comparison of the renal clearance of inulin following a single injection with that during constant infusion, the latter gave values which were 4% higher than the single injection clearance, with a standard deviation of 14% in 19 subjects (Olbrich et al, 1950). However, in this study, the midpoint plasma concentration for each collection period was estimated from the semilogarithmic plot of the inulin plasma concentration against time, and this is less accurate than the method used in the present study (Notari, 1987).

The renal clearance of inulin following a single injection fell progressively after two hours. As the corresponding creatinine clearance did not decrease, a true reduction in glomerular filtration rate during the study seems unlikely. This phenomenon has been reported previously (Josephson & Lindahl, 1943; Ferguson et al, 1950; Laake, 1954; Barnard et al, 1955), but the decline was less marked, and the pharmacokinetic methods used for estimating the clearance were inappropriate. The progressive fall in inulin clearance after 2 hours could be due to factors such as, changing differences in the arterial and venous concentrations of inulin over time, concentration-dependant inulin clearance due to tubular reabsorption at low concentrations, or selective filtration of the lower molecular weight fractions of inulin. These factors are discussed in detail in section III.

The major advantage of the single injection technique is the ability to calculate the clearance from the plasma concentration-time decay curve thus, obviating the major errors associated with urine collection (Brochner-Mortensen, 1985). The plasma inulin concentration-time curve after a single injection showed an initial rapid fall, followed by a slower decreasing rate of decline, which did not appear to become linear until 4-8 hours. The transition from the initial rapid to the slower

phase, occurred at 30-50 minutes. The curvilinear concentration-time curve upto two hours, could be fitted to a two compartmental pharmacokinetic model, as found by other investigators (Broberger, 1973; Fawer et al, 1979; Muller-Suur et al, 1983). However, after two hours, a multi compartmental model is necessary for a proper fit of the data. The total body clearance of inulin also fell after two hours, and subsequent clearances were significantly lower than the 0-2 hour clearance. The fall in total body clearance of inulin after two hours has not been reported previously, because sampling has not been continued after two hours, except in patients with reduced renal function. However, Ladegaard-Pedersen (1972) extrapolated data from the last 60 minutes of a 3 hour curve in volunteers with normal renal function, but found no change after 2 hours.

The pharmacokinetic model used for the calculation of total clearance in this study requires that the clearance stays constant during the period of measurement, and that distribution is complete before extrapolation (Gibaldi, 1984). Distribution equilibrium is said to occur 40-60 minutes after rapid intravenous administration of inulin (Schachter et al, 1950). The result from the present study is in good agreement as the mean distribution half life was 8 minutes. Distribution was therefore more than 95 % complete after 48 minutes. This together with the fall in the renal clearance of inulin after two hours, suggests that the AUC from the 0-2 two hour plasma concentration data extrapolated to infinity would give the best estimate of the total body clearance of inulin. The fall in the rate of elimination, and the total body clearance of inulin after this time, reflects the progressively decreasing renal clearance of inulin after two hours.

The total body clearance of inulin from 0-2 hours was essentially the same, as the 0-2 hour single injection

renal clearance and the constant infusion clearance. The rather weak correlation between the total body clearance and the corresponding renal clearance is surprising, but small numbers were involved. The correlation between the total body clearance of inulin and the corresponding renal clearance for all the single injection studies (N=31) reported in section III, gave a correlation coefficient r, of 0.96, and the regression line was parallel to the line of identity (see Fig 2.3.9, section III). Overall the total body clearance overestimates the renal clearance by 6%.

This overestimate implies limited extrarenal clearance of inulin although, this has not been well investigated. Biliary excretion is thought to be insignificant, and metabolism is not thought to occur (Smith, 1951). Other possible explanations are, underestimation of the area under the plasma concentration-time curve, or non linear-kinetics. One other explanation for the higher total body clearance, is that the injected dose of inulin was too small. This could occur if the polyfructosan chains of the inulin were partially hydrolysed to fructose during the heating, which was necessary in preparation of the solution, prior to administration. On the other hand, the renal clearance of inulin may be an underestimate of the true clearance, due to retention of inulin in the urinary tract. This could occur with residual urine in the bladder. Incomplete bladder emptying is established as the greatest single source, of error, unless catheterization is performed (Zender et al, 1968). For ethical and practical reasons, this was not done in the present study. Rehling et al, (1984), reported a 23 % overestimate of the total body clearance compared to the renal clearance in patients with one kidney, following a single injection, but no catheterization was performed. Overestimation of the total body clearance of inulin during a constant

infusion, compared with the simultaneously measured renal clearance, was also attributed to incomplete bladder emptying (Rose, 1969). However, in studies in which catheterization was performed, the total body clearance of inulin after a single injection did not overestimate the renal clearance during constant infusion (Broberger, 1973; Boineau et al, 1974). In the present study, the volunteers were water loaded, to reduce the effect of residual urine, and incomplete bladder emptying.

Differences in the arterial and venous concentrations of inulin, may be another factor. Venous blood was sampled, but inulin is cleared by the kidney from arterial blood. The venous plasma concentration may be up to 7 % higher than the arterial concentration, two hours after an intravenous injection (Brun et al, 1949; Ferguson et al, 1950). However, as arterial concentrations are higher during the distribution phase, these differences are likely to be cancelled over the whole period.

The rate of excretion of inulin into the bladder lags behind the plasma concentration at a given moment, due to a delay after filtration of the inulin, and its arrival in the bladder. This is more of a problem where plasma concentrations are changing rapidly (Tucker, 1981). Errors can be minimised by, increasing the period of collection and increasing the urine flow rate (Pihl, 1973). In the present study the urine collection periods were an hour, and the mean flow rate for the second hour was 5 ml/min. From the formula of Nosslin (1965), the calculated delay time is 4.1 minutes, which in 60 minutes represents a 7 % error. However, the blood was sampled up to two minutes before the volunteer voided, thus reducing the error to under 4 % .

SUMMARY

The single injection technique for measurement of the total body or renal clearance of inulin, gave results

which were similar to those obtained by the standard constant infusion method, when sampling was restricted to the first two hours. The total body clearance slightly overestimates the renal clearance, probably because of problems with urine collection. The ability to measure the total body clearance simply and accurately, is a distinct advantage, and a reason for the popularity of single injection techniques using radioactive markers. In healthy individuals estimation of the GFR by the inulin single injection technique, is easy, and the risks inherent with the use of radioactivity are avoided. The technique is simple to carry out, lasts only two hours and there is minimum discomfort. The only drawback, is that numerous blood samples are needed to ensure an accurate analysis of the concentration-time decay curve, following a single injection.

SECTION II

REPRODUCIBILITY OF THE SINGLE INJECTION TECHNIQUE FOR ESTIMATION OF THE INULIN CLEARANCE

INTRODUCTION

As described in the last section, inulin is suitable for the measurement of GFR over the first two hours, after a rapid intravenous bolus injection. The usefulness of any technique for measurement of the GFR depends on its reliability, accuracy and reproducibility (Brochner-Mortensen & Rodbro, 1976a). The former was discussed in the last section, and in this section the results of repeated estimates of the GFR in the same individual over time, are presented. Data obtained by the constant infusion method over a similar period, were kindly provided by Dr T. MacDonald, allowing a comparison between the classical and the single injection methods.

METHODS

Single injection

Eight healthy male volunteers with a mean age 27 ± 3 years (range 24-32 yrs) weighing 70 ± 10 Kg (range 56-85 Kg) received inulin intravenously, by the single injection technique, on 3 different occasions, at intervals of 6 to 22 days between the first two study days, and 7 days between the second and third studies. The experimental conditions were similar to those described on page 50 except that, the fluid intake was a 100 ml of water every half hour for the duration of the study (2 h). The same batches of inulin (7 subjects) and "Inutest", (one subject) were used for each volunteer, on each occasion.

Constant infusion method

Nine healthy male volunteers with a mean age 30 ± 5 years (range 22-36 yrs) weighing 69 ± 7 Kg (range 56-81 Kg) received "Inutest", by the classical infusion method, on 3 different occasions at intervals of 7 to 15 days between the first two study days in eight subjects, and a period of 5 months in one subject. The interval between

the second and third studies, ranged from 4 to 28 days. The experimental conditions were similar to those described on page 49 except that, the fluid intake was 200 ml every half hour for 3 hours, and the urine collection periods were for one hour. The same batch of "Inutest" was used for each volunteer on each occasion.

Sample collection and analysis for inulin were as previously described (pages 50 & 51).

Data analysis

Single injection

The total body and renal clearances of inulin following a single injection were calculated as described previously (pages 52 & 53). The renal clearance was estimated from the 0-2 hour plasma and urine data, whilst the total body clearance was calculated from the 0-2 hour plasma data, extrapolated to infinity.

Constant infusion

The renal clearance of inulin estimated by constant infusion was calculated as described on page 52. The values reported are the mean of the first two collection periods after equilibration.

All clearances were corrected for surface area as described on page 54.

Statistical methods

The coefficients of variation for the repeated estimates were calculated for each subject, as described on page 52. The statistical significance of differences between mean clearances over the three studies, were estimated by two way analysis of variance. A non-parametric Wilcoxon rank sum test was used for differences in the coefficients of variation of the two methods. The null hypothesis was rejected when $p < 0.05$.

RESULTS

The individual total body and renal clearances of inulin following a single injection on three different days are given in Tables 2.2.1A & B, and the results for the constant infusion method are given in Table 2.2.2. The individual plasma and urine concentrations for the single injection are given in Appendix II.

The mean total body clearances of inulin following a single injection for days 1, 2, and 3 were 106, 104, and 106 ml/min/1.73 m^2 and, the corresponding renal clearances were 97, 98, and 98 ml/min/1.73 m^2 respectively. The mean renal clearances of inulin for the 3 days using the constant infusion technique were 104, 99, 91 ml/min/1.73 m^2. There were no significant changes in the mean clearances over the 3 days with any of the methods.

The reproducibility of each method on the 3 separate days is expressed as the coefficient of variation. The individual coefficients of variation are given in Table 2.2.1A & B for the single injection total body and renal clearances, and Table 2.2.2 for the renal clearance by constant infusion. The smallest mean and individual ranges of variation, were found with the total body clearance method (mean coefficient of variation 4.6% range 1.0-8.7%). The variation in the mean renal clearance by single injection was 7.5%, range 1.0-11.7%. In contrast, the mean variation in clearance determined by constant infusion was 15.1% (range 4.0-32.6%). The coefficient of variation for the total body clearance of inulin following single injection, was significantly less than for the two renal clearance methods ($p < 0.01$). There were no significant differences between the two renal clearance methods.

TABLE 2.2.1A

The total body clearance of inulin (ml/min/1.73 m^2) determined in healthy male volunteers by the single injection technique, on three different days

	STUDY DAY					
SUBJECT	1	2	3	MEAN	±SD	CV %
GM	97	95	96	96	1.0	1.0
EC	95	92	86	91	4.6	5.0
AB	114	97	106	106	8.5	8.0
RJ	114	96	103	104	9.1	8.7
AD	117	120	122	120	2.5	2.1
JA	99	107	104	103	4.0	3.9
SA	117	116	124	119	4.4	3.7
MS	98	107	103	103	4.5	4.4
MEAN	106	104	106	105	4.8	4.6
± SD	9.9	10.4	12.5	10.0	2.7	2.7

TABLE 2.2.1B

Renal clearance of inulin (ml/min/1.73 m^2) in the same subjects on the same three days, as in Table 2.2.1A.

	STUDY DAY					
SUBJECT	1	2	3	MEAN	±SD	CV %
GM	84	96	86	89	6.4	7.3
EC	99	98	82	93	9.5	10.3
AB	98	80	81	86	10.1	11.7
RJ	103	85	103	97	10.4	10.7
AD	116	133	146	132	15.0	11.4
JA	95	103	99	99	4.0	4.0
SA	96	97	95	96	1.0	1.0
MS	85	90	90	88	2.9	3.3
MEAN	97	98	98	98	7.4	7.5
± SD	10.1	16.1	21.0	14.5	4.7	4.2

TABLE 2.2.2

The renal clearance of inulin (ml/min/1.73 m^2) determined in healthy male volunteers by the constant infusion method, on three different days

	STUDY DAY					
SUBJECT	1	2	3	MEAN	±SD	CV %
DM	121	102	100	108	11.6	10.8
BB	90	95	109	98	9.8	10.0
AD	97	94	114	102	10.8	10.6
DP	99	106	99	101	4.0	4.0
KH	70	93	102	88	16.5	18.7
AK	96	101	83	93	9.3	10.0
SM	81	96	97	91	9.0	9.8
BW	111	98	65	91	23.7	26.0
JM	175	108	99	127	41.5	32.6
MEAN	104	99	96	100	15.1	14.7
± SD	30.4	5.4	14.5	12.0	11.3	9.2

DISCUSSION

The reproducibility of the total body and renal clearances of inulin on three separate occasions, using the single injection method, has been evaluated and compared with the renal clearances of inulin determined by the constant infusion method, over a similar period. The latter is the reference method, but it has many disadvantages. A method for routine clinical use has to be simple, accurate and reproducible; it is the latter which was the concern of this study. The results show that there was significantly less variation in the total body clearance of inulin following a single injection, than the renal clearances determined by both single injection, and constant infusion methods. The renal clearance by single injection also varied less than the renal clearance during constant infusion, but the difference was not statistically significant.

Variability in clearance measurements has three main components, a) true variation in the GFR, b) analytical variability, and c) errors in urine collection.

a) Glomerular filtration is maintained by auto-regulation, at a relatively constant rate in healthy individuals, over a wide range of arterial blood pressures (Baylis, 1986). Certain physiological factors such as stress, exercise and pregnancy alter GFR, and there is also a diurnal variation (O'Connor, 1981). The subjects in this study were not affected by these factors, with the possible exception of stress, and they were studied under the same conditions, at the same time of day, on each occasion. Variation in the GFR itself is unlikely to be the main contributing factor to the differences between the methods, as both total body and renal clearances would have been affected equally, after a single injection.

b) Analytical variability is also unlikely to explain the differences in variation between total body and renal

clearance as, both plasma and urine concentrations of inulin, were well above the limit of detection, and the coefficient of variation of the method for repeated samples carried out within the medical renal unit laboratories, is about 5%.

c) Errors in urine collection are the most likely explanation for the greater variability in the renal clearance, than the total body clearance. These arise mainly from inaccurate timing, and incomplete urine collections. In another study, the inter-assay coefficient of variation for the constant infusion was 1.7 %, while the error of measurement of the urine flow rate was 7.1 % (Zender et al, 1968). Thus, urine collection is likely to be the single greatest source of variation in the measurement of the renal clearance of inulin. Urine collection may be incomplete without catheterization (as in this study) and this may contribute to the wide individual range observed. Sudden changes in urine flow may have a similar effect. Thus, methods which do not depend on urine collection, are likely to be more reproducible, than those which do.

The most widely used routine clinical measure of the GFR is the endogenous creatinine clearance obtained, using either 24 hour urine collections, or estimated by nomogram, from the plasma creatinine concentration. These methods have coefficients of variation of 25 % and 15 % respectively (Brochner-Mortensen & Rodbro, 1976a). The coefficients of variation for the inulin clearance following a single injection, were less in the present study than reported for the creatinine clearance, and of the same order as with radiolabelled markers (Brochner-Mortensen, 1985). The day to day variation in the total body clearance of inulin, was less than the period to period variation, for the constant infusion method (Kennedy & Kleh, 1953).

SUMMARY

The total body and renal clearances of inulin, following a single intravenous bolus, in 8 healthy male volunteers, were estimated on three separate occasions in the same person. This was compared to the renal clearance of inulin, during constant infusion of inulin, over a similar period. The inulin clearance measured by the single injection technique is reproducible on repeated estimations and less variable than the constant infusion method. There is least variation with total body clearance measurements as urine collections are not required.

SECTION III

MECHANISMS OF THE PROGRESSIVE FALL IN INULIN CLEARANCE AFTER A SINGLE INTRAVENOUS INJECTION

INTRODUCTION

The initial studies reported in section one showed that, the mean renal clearance of inulin remains relatively constant for the first two hours after an intravenous bolus injection but, after this time, the clearance of inulin falls progressively as the plasma concentration declines. The corresponding creatinine clearance did not show a similar decrease, and therefore, a true reduction in GFR is unlikely. This was unexpected, as the rate of excretion of inulin is thought to be directly proportional to its plasma concentration over a wide range of plasma concentrations (50 to 4000 mg/l; Shannon & Smith, 1935; Miller et al, 1940).

However, a fall in the renal clearance of inulin has been reported previously. Josephson and Lindahl (1943), first noted that after an intravenous bolus of inulin, the renal clearance estimated in the third collection period, was significantly lower than the first two, and similar observations were reported by Laake (1954). Others have reported a fall almost immediately after the injection (Ferguson et al, 1950; Barnard et al, 1955). The reasons suggested for this decline in inulin clearance include, tubular reabsorption, selective filtration (Ferguson et al, 1950; Barnard et al, 1955), and a decrease in extracellular fluid tonicity and thus, intrarenal pressure due to fluid loading (Laake, 1954). Ferguson et al (1950), infused inulin at different rates, and reported that at different plasma concentrations, the clearance of inulin differed. However, a decline in renal clearance has not been observed by others using single injection methods (Alving & Miller, 1940; Carrie et al, 1980), or during clearance measurements at different steady state plasma concentrations of inulin produced by stepping up, or down the infusion rate (Kennedy & Kleh, 1953; Cole et al, 1972). There was no evidence to support tubular reabsorption of inulin or plasma concentration

dependant clearance.

The early studies which reported the fall in inulin clearance used inappropriate pharmacokinetic analysis, and there have been no subsequent reports using appropriate kinetics which indicate a fall in inulin clearance. This may be due to the fact that in the most recent reports, the estimated clearance was based on the total body clearance of inulin, or the studies have been carried out in patients with reduced renal function (Broberger, 1973; Muller-Suur et al, 1983; Rehling et al, 1984). It should be noted that, the validity of the pharmacokinetic models used to calculate total body clearance depends on the assumption that, the clearance of the measured marker remains constant, throughout the period of measurement (Gibaldi, 1984). The results presented in section I clearly show a fall in the renal clearance of inulin and therefore, measurements of the total body clearance made during the period of declining renal clearance, are invalid.

The fall in inulin clearance could be due to a number of factors including, 1) changing differences between the arterial and venous plasma concentration of inulin over time, 2) concentration dependant inulin clearance due to tubular reabsorption, 3) selective filtration of the low molecular weight fraction of inulin or 4) an increasing proportion cleared extrarenally, at low plasma concentrations.

The possible role of these factors has been investigated by further analysis of the results of studies in 23 males with normal renal function, and 8 patients with reduced renal function following an intravenous bolus of inulin. The renal clearance of inulin was assessed in relation to, time, plasma concentration, urine flow rate, and amount recovered in 24 hours. In addition, the renal clearance of inulin has been investigated under steady state conditions, at

different plasma concentrations in 8 male subjects with normal renal function. The endogenous creatinine clearance was also measured, to control for naturally occurring fluctuations in GFR.

METHODS

Study one. Single injection in 23 healthy males.

Single injection studies were carried out in 23 healthy male volunteers aged 21-32 years (mean 26 ± 3 yrs), weighing 57-89 Kg (mean 71 ± 8 Kg). The protocol and experimental conditions were the same as described in section I (p 50), except that in 19 volunteers the fluid intake during the study was reduced from 130 ml to 100 ml every half hour, for 6-8 hours. Inulin (Kerfoot, Barnsley, U.K., 5 g in 50 ml, 10% w/v) was used, except in volunteers JN, RF, and MS who received "Inutest" (Lavevosan Gesellschaft, Austria, 5 g in 20 ml, 25% w/v, this was diluted with 28 ml of normal saline and administered over 5 minutes).

Study two. Single injection in 8 patients with renal impairment.

Single injection studies were carried out in 8 patients with decreased glomerular function (range 20-70 ml/min/1.73 m^2), as assessed by an eight hour creatinine clearance. 6 males and 2 females with a mean age of 59 ± 7 years (range 46-65 yrs) weighing 61-88 Kg (mean 74 ± 10 Kg) were entered into the study, which was approved by the local ethical committee. The protocol and experimental conditions were as described in section I (p 50) with the following exceptions:- a) The number of blood samples were reduced by excluding the following time points 40, 50, and 75 minutes. A 45 minute sample was added. b) The fluid intake was at the rate of 150 ml every half hour for the first four hours thereafter, it was ad libitum. All patients received the same batch of

"Inutest" (Lavevosan Gesellschaft, Austria. 5 g in 20 ml, 25% w/v). This was diluted with 28 ml of normal saline and administered over 5 minutes.

Study three. Incremental infusion in 8 healthy males.

Eight healthy male volunteers aged between 25-42 years (mean age 30 ± 6 yrs), weighing 55-77 Kg (mean 69 ± 7 Kg), received two constant infusions of inulin ("Inutest"), in random order on different days. The same batch was used throughout the study. The constant infusion was intended to give a stepwise increase in plasma concentrations of inulin on one occasion, and a stepwise decrease on the other. The interval between the two studies ranged between 7 and 22 days in seven subjects, and was six months in the other. The volunteers fasted from 22.00 hours and, on the morning of the study drank 500 ml of water, an hour before the study commenced, followed by 150 ml every half hour for 7½ hours. The subjects were supine for the duration of the study, but stood to empty their bladder. No food or caffeine containing beverages were allowed during the study.

The initial procedure for the measurement of inulin clearance by constant infusion, was as described in section I (p 49). On "step up" days, the subjects were given an initial loading dose of "Inutest" 375 mg (1.5 ml). The infusion contained "Inutest" (1679 mg/ml in 300 ml of 0.9% saline), which was given at the rate of 336 mg/min. After equilibration for an hour, the subjects emptied their bladder and blood was drawn at the beginning and end of three half hour urine collection periods. At the end of the third period, another loading intravenous injection of "Inutest" was administered (375 mg, 1.5 ml), and the infusion rate was increased to 672 mg/min. Four half hour urine collections were then made with blood sampling, as described above. At the end of the fourth collection period, another intravenous

injection of "Inutest" was given (1125 mg, 4.5 ml) and the infusion rate was increased to 2015 mg/min. Urine was collected with blood sampling for 4 final half hour periods, as before (Fig 2.3.1).

On "step down" study days, the volunteers received an initial loading dose of "Inutest" (3000 mg, 12 ml), prior to the infusion, which was maintained at a rate similar to that at the end of the "step up" study (1913 mg/min). Blood was sampled and urine collections were also made at the same times, as during the "step up" study. However, at the times when an intravenous bolus injection of inulin was given in the "step up" study, the infusion was stopped for 15 minutes, after which the infusion rate was reduced to 638 mg/min for the middle, and 319 mg/min for the final periods (fig 2.3.1).

The blood sampling cannula was kept open by flushing with 1 ml of heparinised saline (10 units/ml), after the withdrawal of each sample. Ten ml of the infused solution was collected at the end of each study, for measurement of the actual inulin concentration.

Sample collection and analysis

Blood and urine samples were collected as described, and inulin and creatinine estimated by the same procedures, as described previously (Section I, p 50 & 51).

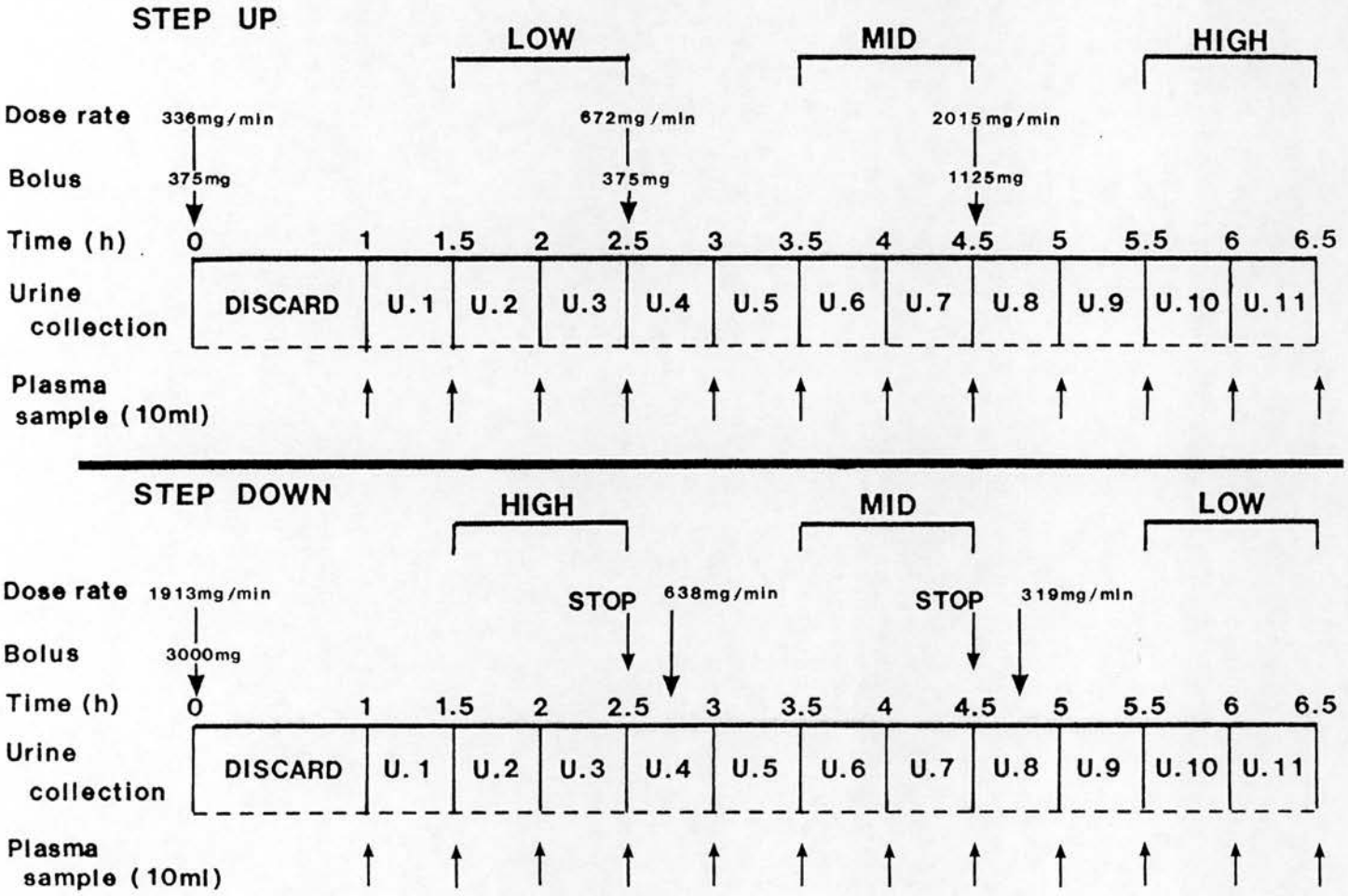
Data analysis

Study one.

The total body and renal clearances of inulin, and the renal clearance of creatinine were calculated for the same periods, and in the same way, as described in section I (p 52-54). The mean distribution half life was calculated from the constants obtained by pharmacokinetic analysis of the plasma concentration-time curve, up to 2 hours. From the best fit of the data, using the "multi"

Fig 2.3.1

Plan of step up and step down constant infusion studies with inulin



non-linear regression analysis programme (Yamaoka et al, 1981), the rate constant was obtained for the initial rapid fall in plasma concentration, which represents the distribution phase. The mean half life of distribution was then obtained by dividing ln 2 by the distribution rate constant.

The percentage of the dose recovered in each urine collection period was calculated by dividing the amount excreted by the administered dose x 100.

The midpoint plasma concentration for the plot of urinary excretion rate against plasma concentration, was calculated by dividing the actual area under the plasma concentration time curve for each collection period by the duration.

Study two.

The total body and renal clearances of inulin and the renal clearance of creatinine were calculated, as described in section I (p 52-54), and the mean distribution half life was estimated, as described in study one over four hours.

The percentage of dose recovered in each urine collection period was calculated, as above, for the period 0-8 hours, as no 8-24 hour collection was made.

Study three.

Because of the need to allow time for equilibration after changing the rate of administration of inulin, the results for the first urine collection period, after each change of dose (U1, U4, U5, U8, and U9 see Fig 2.3.1), have been disregarded. The samples were pooled to represent one hour periods from 1½ to 2½ hours, 3½ to 4½ hours and 5½ to 6½ hours. In the "step up" study, these successive time periods are referred to as "low", "mid", and "high" dose infusions, and the reverse applies for the "step down" study (Fig 2.3.1).

The renal clearance of inulin and creatinine were

calculated, as described previously, for the constant infusion method (section I, p 52).

The total body clearance of inulin (TBC) was calculated from the following relationship :-

$$TBC = \frac{\text{rate of infusion}}{Css} \qquad \text{(Equation 5)}$$

where Css is the steady state plasma concentration of inulin calculated from the mean of the last two inulin plasma concentration values, during each infusion step.

The percentage urinary recovery of inulin was calculated by dividing the urinary excretion rate of inulin of the last two periods in each infusion step, by the infusion rate.

All clearances were corrected for body surface area of 1.73 m^2 as described on page 54 section I.

Statistical analysis

Statistical analysis were carried out between collection periods, using two way analysis of variance (ANOVA). In addition, the significance of differences between means were determined using a students two tailed "t" test, and the null hypothesis was rejected if $p < 0.05$. Correlation coefficients were calculated by linear regression analysis.

RESULTS

Plasma concentrations

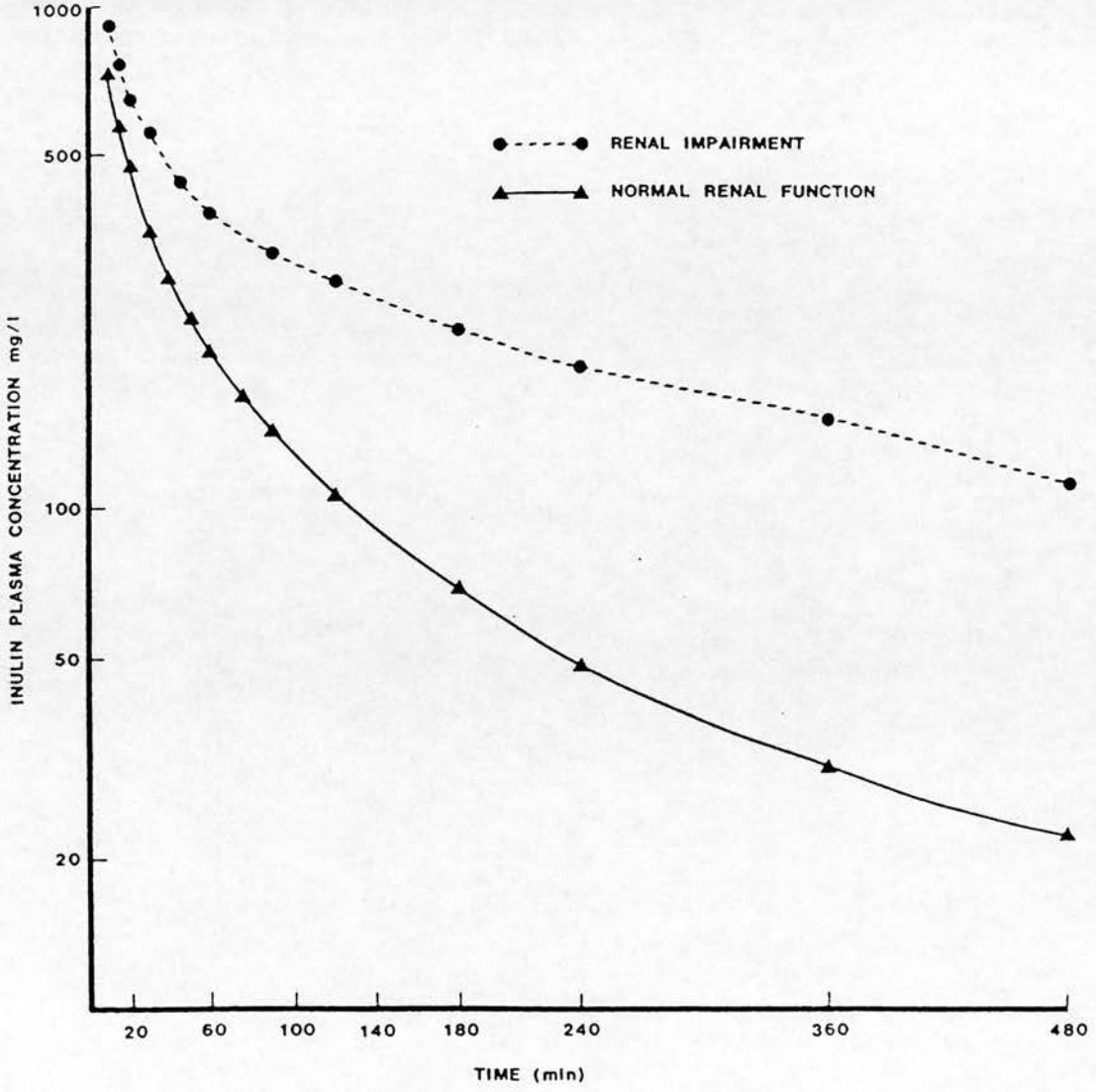
The plasma concentrations of inulin for each individual in each study, are given in Appendix I, III & IV for study one, two and three respectively.

Study one.

Following intravenous bolus administration, the plasma concentrations of inulin fell rapidly in all subjects in a curvilinear manner, when plotted semilogarithmically against time (Fig 2.3.2). The plasma concentration-time data up to two hours could be fitted

Fig 2.3.2

The mean plasma concentration-time curves following an intravenous bolus of inulin in 23 healthy males, and 8 patients with impaired renal function.



compartment model, but a terminal linear phase was not seen. The mean plasma inulin concentrations declined from 736 ± 92 mg/l at 10 minutes, to 22 ± 13 mg/l at 480 minutes. The mean distribution half life was 7.7 minutes, therefore distribution was on average greater than 95 % complete, 46 minutes after the single injection.

Study two.

Following intravenous bolus administration, the plasma concentrations of inulin fell again in all subjects in a curvilinear manner, when plotted semilogarithmically against time (Fig 2.3.2). The plasma data was consistent with a three compartment model up to eight hours, and the plasma concentration-time relationship appeared to be log-linear from 4-8 hours. Up to four hours, the data could be fitted to a two compartment model. The mean inulin plasma concentrations declined from 921 ± 127 mg/l at 10 minutes, to 111 ± 46 mg/l at 480 minutes. The mean distribution half life was 13.8 minutes, therefore, distribution was on average more than 95 % complete, 83 minutes after administration.

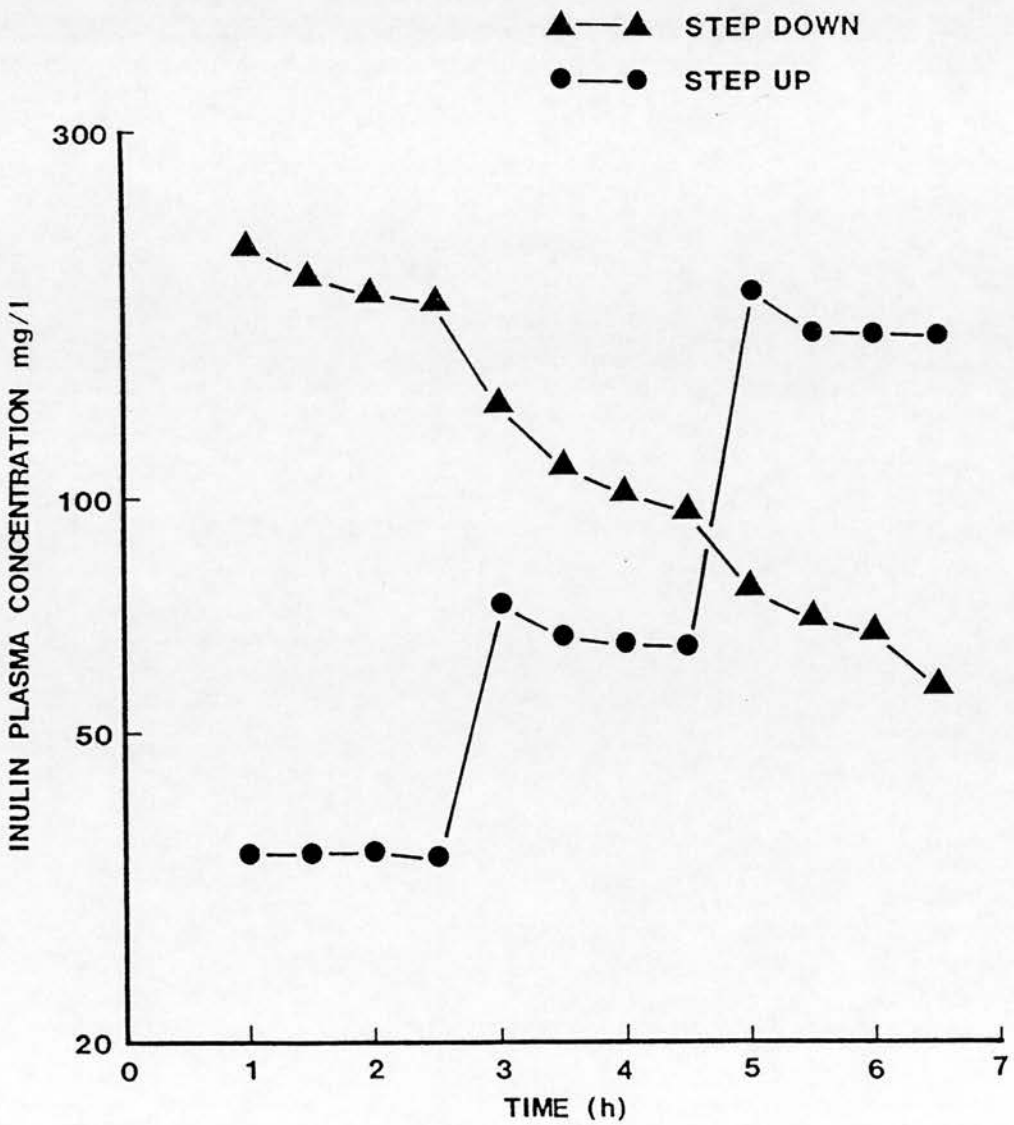
Study three.

The mean plasma concentrations of inulin during each "step up" and "step down" infusion are shown in Fig 2.3.3. With the "step up" infusion, the plasma concentrations appeared to reach steady state during the low, mid, and high periods, at the times when the clearance of inulin, was measured. The respective mean midpoint plasma inulin concentrations were 35, 65.6, and 165 mg/l.

With the "step down" study, the mean midpoint plasma inulin concentrations were 187, 103.2, and 65 mg/l but, steady state was not achieved, during any of the collection periods.

Fig 2.3.3

A plot of the mean inulin plasma concentrations achieved during the step up and step down constant infusion of inulin in 8 males with normal renal function.



The relationship between the renal clearance and plasma concentration of inulin

Study one.

The individual renal clearances of inulin for each collection period are given in Table 2.3.1. The mean renal clearance of inulin over the first and second hours was similar, but thereafter it fell progressively by 7, 14, 36, and 50 ml/min/1.73 m^2 for 2-3, 3-4, 4-6, and 6-8 hour periods respectively, relative to the clearance measured from 0-1 hours. The sequential mean renal clearances of inulin were 97, 100, 90, 83, 61 and 47 ml/min/1.73 m^2 (Fig 2.3.4). This fall in the renal clearance of inulin over time and, with falling plasma concentrations, was statistically significant ($p< 0.01$). The 0-1 hour renal clearance of inulin was significantly greater than for any period after 2 hours ($p< 0.03$ for 2-3 h and $p< 0.001$ for 3-4, 4-6, and 6-8 h).

A plot of the mean renal clearance of inulin against the mean midpoint plasma concentration for each collection period shows, that the clearance remained relatively constant at plasma concentrations ranging from 150 to 480 mg/l. However, as the concentration of inulin declined below 100 mg/l, the renal clearance fell progressively (Fig 2.3.5).

Study two.

The individual renal clearances of inulin for each collection period are given in Table 2.3.2A. The fall in the mean renal clearance of inulin was not as dramatic as in the healthy subjects. The clearance over the first and second hours was similar, after which time there was a small, but progressive fall of 6, 14, 14, and 19.4 % for 2-3, 3-4, 4-6, and 6-8 hours periods respectively, relative to the clearance from 0-1 hours. The mean renal clearances of inulin were 36, 37, 34, 31, 31, and 29 ml/min/1.73 m^2, for 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 hours

TABLE 2.3.1

Renal clearance of inulin (ml/mim/1.73 m^2) after a single intravenous injection of inulin in 23 healthy male subjects

	COLLECTION PERIOD (hours)					
SUBJECT	0-1	1-2	2-3	3-4	4-6	6-8
PF	108	102	105	120	97	72
GS	87	88	79	72	41	26
JN	78	61	59	57	40	30
DM	109	104	74	56	39	37
MK	90	99	98	63	49	28
CP	89	90	69	73	66	44
BH	72	82	68	65	43	25
BS	94	93	76	74	53	34
PL	123	108	103	95	64	64
TM	110	117	105	100	82	44
GW	81	90	80	75	66	46
AT	106	146	123	101	81	128
EC	101	95	96	101	77	25
WW	93	90	92	85	64	75
AB	105	94	99	98	72	50
RJ	104	100	79	90	67	43
AD	117	113	115	106	68	51
JA	95	97	79	61	64	65
RF	108	102	85	92	70	43
PD	83	130	117	82	24	13
MS	87	78	76	70	49	55
SA	98	91	103	81	64	51
JG	104	132	88	86	71	38
MEAN	97	100	90	83	61	47
±SD	13	19	17	17	17	24

Fig 2.3.4

Renal clearances of inulin and creatinine following a single injection of inulin in 23 healthy male subjects. Bars = SD.

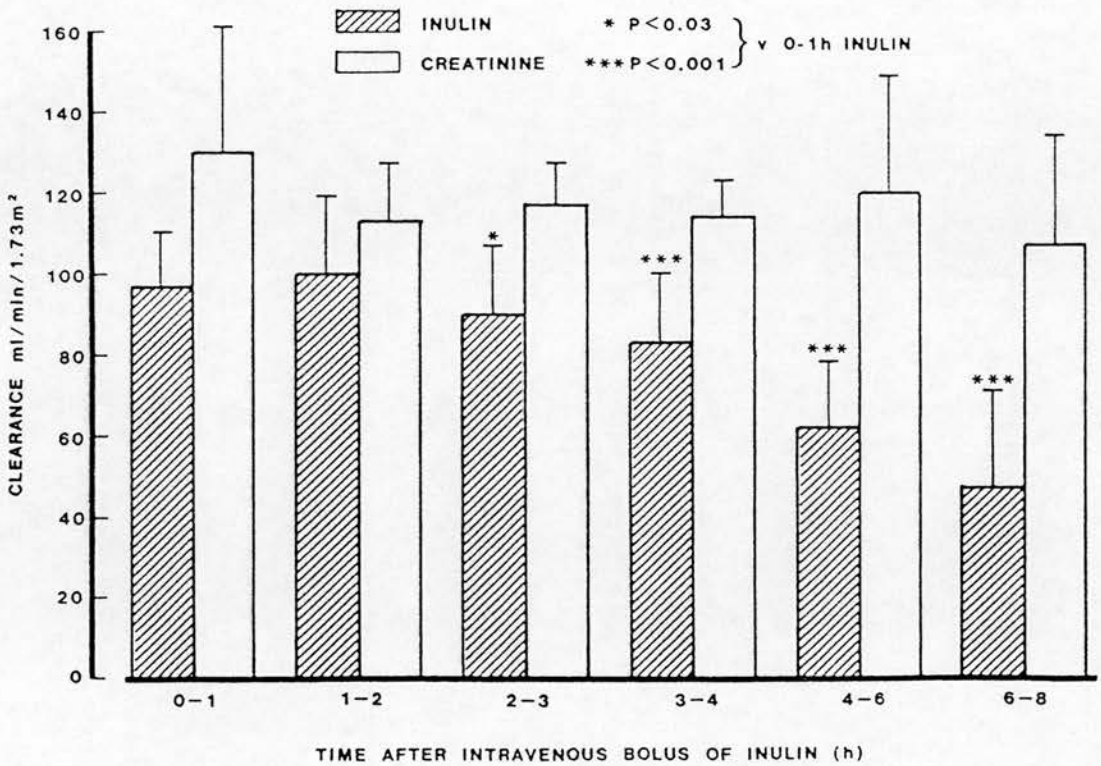


Fig 2.3.5

Relationship of mean inulin renal clearances to mean plasma concentrations in 23 subjects, with normal renal function, and 8 patients with impaired renal function, following a single intravenous injection of inulin. Bars = ± SD.

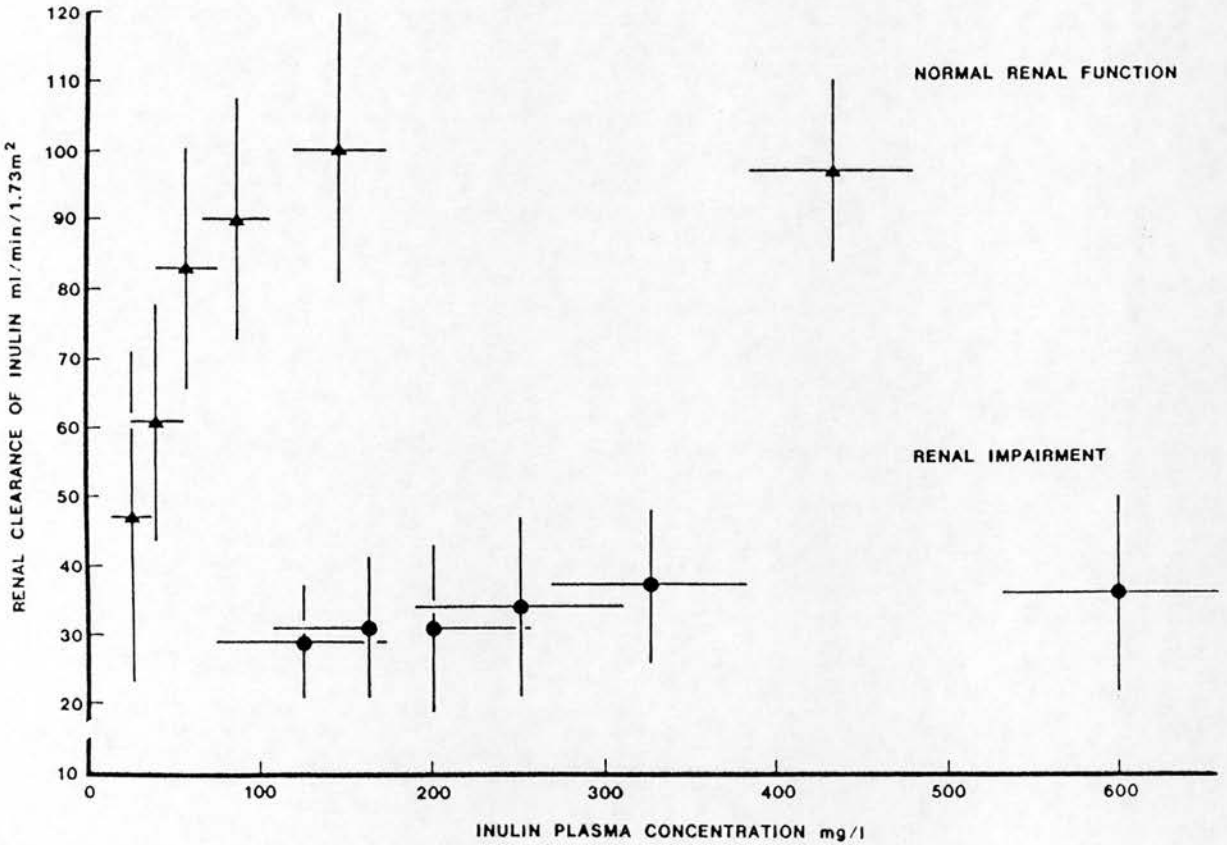


TABLE 2.3.2A

Renal clearance of inulin (ml/min/1.73 m^2) following an intravenous bolus of inulin in 8 renal impaired patients.

	COLLECTION PERIOD (hours)					
SUBJECTS	0-1	1-2	2-3	3-4	4-6	6-8
JB	22	24	23	21	19	18
EC	17	25	22	22	18	23
AD	35	38	35	32	31	29
EH	30	28	27	25	22	22
WA	49	50	54	53	44	43
JH	58	55	51	46	41	37
CP	42	37	36	20	37	30
EB	33	38	22	25	33	29
MEAN	36	37	34	31	31	29
±SD	14	11	13	12	10	8

TABLE 2.3.2B

Total body clearance of inulin (ml/min/1.73 m^2) following an intravenous bolus of inulin in 8 renal impaired patients.

	COLLECTION PERIOD (hours)			
SUBJECTS	0-2	0-4	0-6	0-8
JB	34	24	19	21
EC	26	24	21	18
AD	27	35	30	25
EH	33	26	20	21
WA	56	50	44	43
JH	63	55	49	48
CP	37	43	38	34
EB	43	37	34	29
MEAN	40	37	32	30
±SD	13	12	11	11

respectively (Fig 2.3.6). This fall was significantly related to time and plasma concentration, up to 8 hours ($p< 0.05$), but not up to 4 hours. The clearances during the 4-6 and 6-8 hour periods were significantly lower than from 0-1 hours ($p< 0.05$) and 1-2 hours ($p< 0.005$), and the clearance from the 3-4 hour period was significantly less than from 1-2 hours ($p<0.05$). A plot of the mean renal clearance of inulin against the mean midpoint plasma concentration for each collection period, showed there is a small fall in clearance as the plasma concentration declines from 592 to 126 mg/l (Fig 2.3.4).

Study three.

The renal clearances of inulin are shown in Table 2.3.3A and Fig 2.3.7. During the "step up" infusion the mean inulin clearance increased progressively from 90, to 97 at low and mid, and to 114 ml/min/1.73 m^2 at high plasma concentrations. This rise in clearance was significant over time and increasing plasma concentrations ($p< 0.01$), and the clearances at low and mid, were significantly lower than that at the high plasma concentration ($p< 0.005$). Similarly, the clearance at the low period was significantly lower than that at the mid period ($p< 0.05$).

Conversely, during the "step down" study, the mean inulin clearances were 112, 114, and 94 ml/min/1.73 m^2 for high, mid and low plasma concentrations respectively. This fall in clearance was significant over time and declining plasma concentrations ($p< 0.05$), and the clearance at the low plasma concentration was significantly less than that at the high ($p< 0.002$) and mid ($p< 0.01$) plasma concentrations.

A plot of mean renal clearance of inulin against the mean midpoint plasma concentrations (Fig 2.3.8) for each period shows clearly that, in the "step up" study, the clearances rise as the plasma concentration increases. At both the low and mid periods, the mean plasma concent-

Fig 2.3.6

Renal clearances of inulin and creatinine following a single injection of inulin, in 8 patients with renal impairment. Bars = SD.

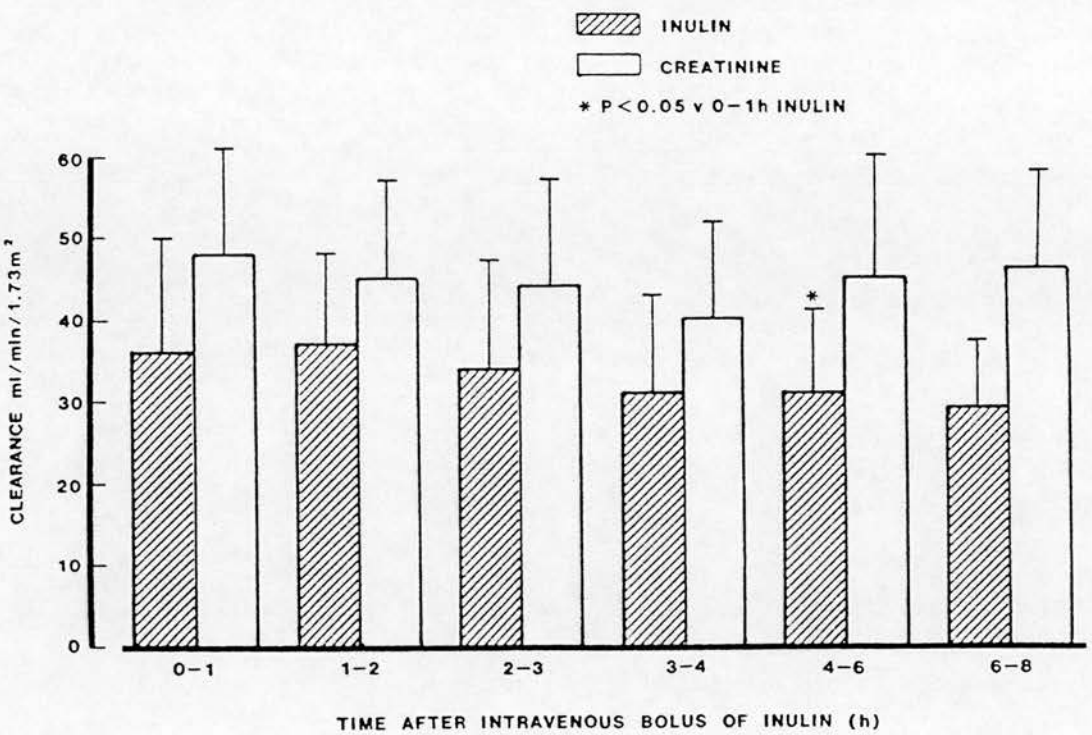


TABLE 2.3.3A

Renal clearance of inulin (ml/min/1.73 m^2) following incremental changes in the dose rate in 8 healthy male subjects

	COLLECTION PERIOD					
	STEP UP			STEP DOWN		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
JN	100	105	136	92	86	76
GS	74	73	97	94	86	72
SB	99	96	110	119	133	114
AD	123	139	133	127	120	119
BB	91	95	121	122	119	86
WW	69	91	99	139	176	126
SM	80	87	103	87	80	68
BW	81	88	114	116	108	94
MEAN	90	97	114	112	114	94
±SD	18	19	15	19	32	23

TABLE 2.3.3B

Total body clearance of inulin (ml/min/1.73 m^2) during step up constant infusion in 8 healthy male subjects

	COLLECTION PERIOD		
	STEP UP		
SUBJECT	LOW	MID	HIGH
JN	117	111	154
GS	88	88	107
SB	114	109	135
AD	129	140	142
BB	85	94	117
WW	70	93	109
SM	80	87	104
BW	84	87	102
MEAN	96	101	121
±SD	21	18	20

Fig 2.3.7
Mean inulin and creatinine renal clearances during step up and step down constant infusions of inulin in 8 healthy males. Bars = SD.

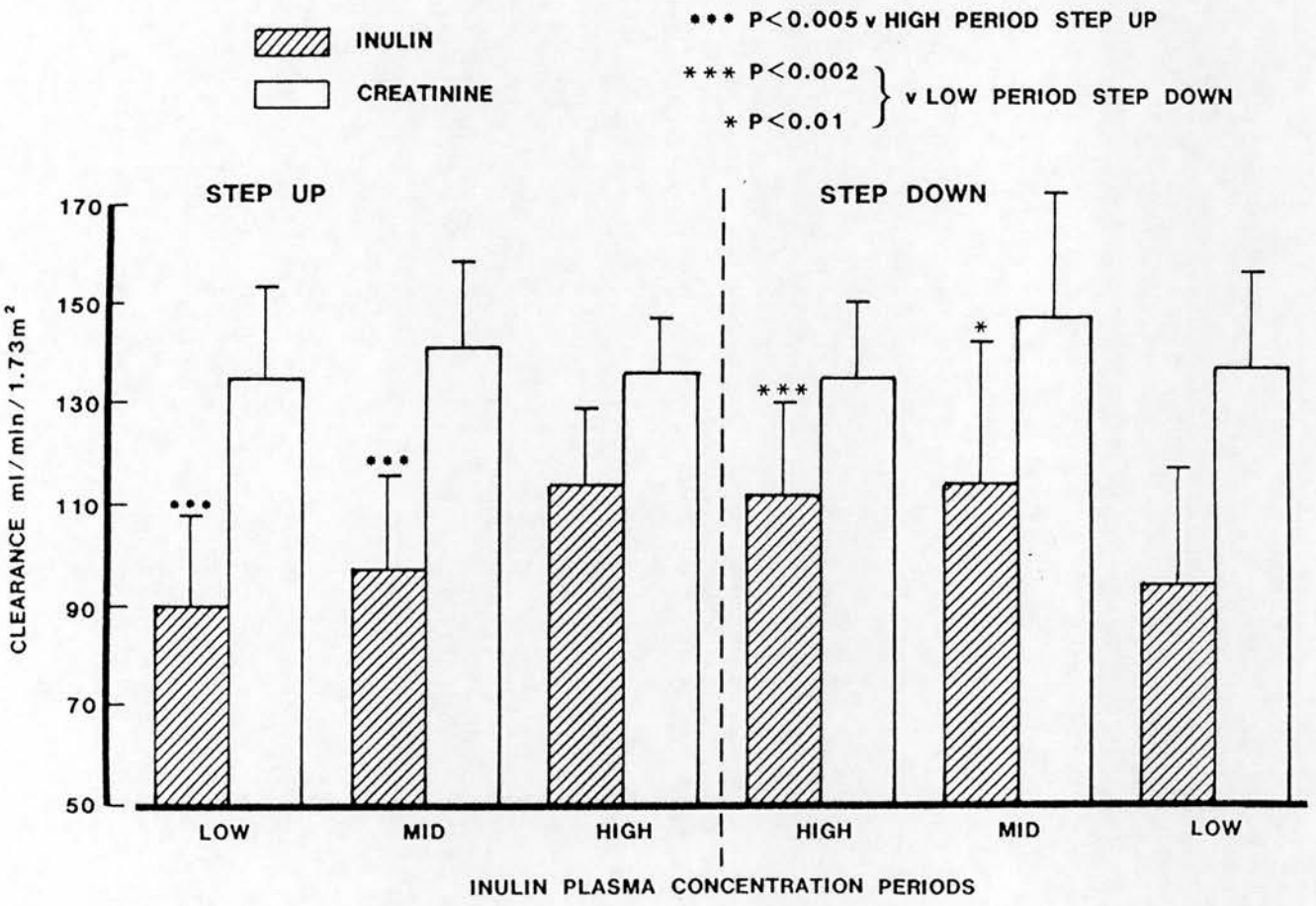
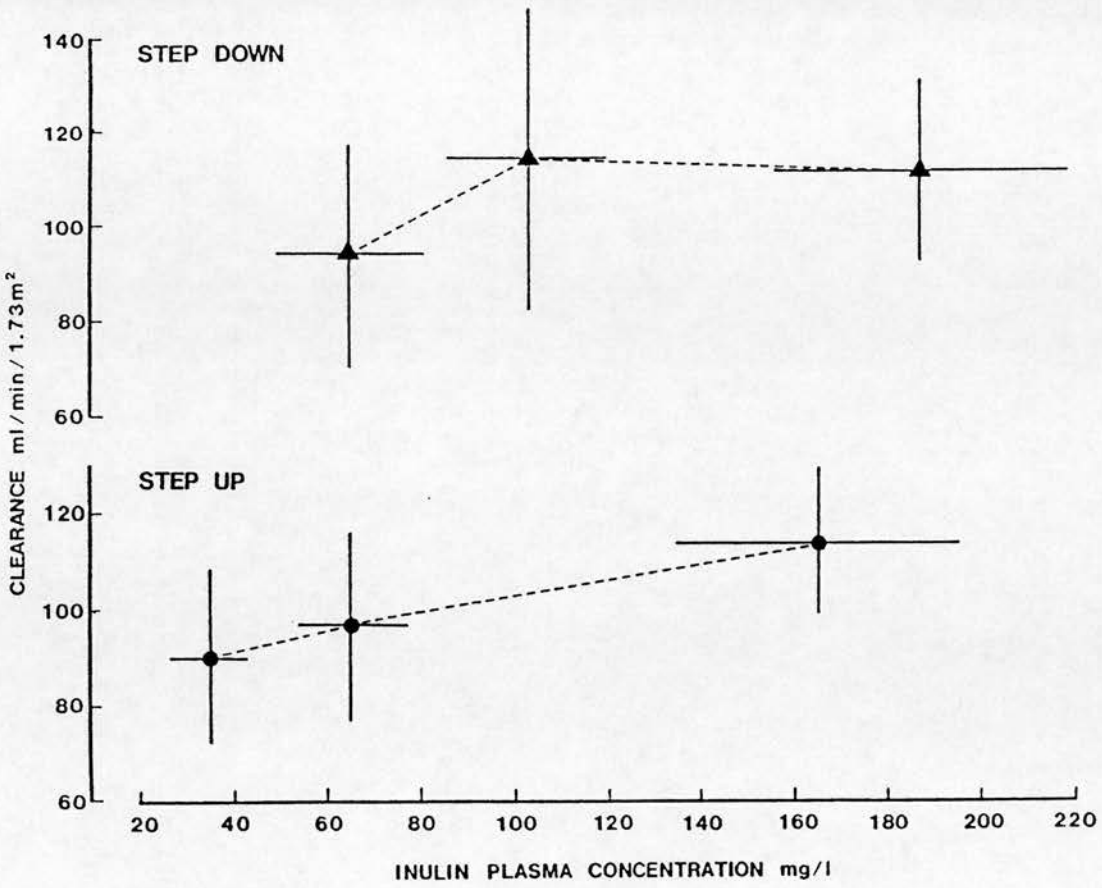


Fig 2.3.8

Plot of inulin renal clearance against plasma concentration of inulin, on step up (bottom), and step down (top) constant infusions, in 8 healthy males. (Mean ± SD).



rations are below 100 mg/l. In the "step down" study, the renal clearance remains similar (in most cases) over the mid to high concentrations, but falls in the low period, when the plasma concentrations are below 100 mg/l (Fig 2.3.8).

Total body clearance of inulin

Study one.

The individual total body clearances are given in Table 2.3.4. The total body clearance of inulin showed a significant fall over time and plasma concentration ($p<0.01$). The total clearance determined from time points after two hours was significantly less, than the total clearance from 0-2 hours ($p<0.001$). The mean total body clearances were 105, 93, and 82 ml/min/1.73 m^2 for 0-2, 0-4, and 0-6 hours respectively.

Study two.

The individual total body clearances are given in Table 2.3.2B. The total body clearance of inulin showed a significant fall over time and plasma concentration ($p<0.01$). The total clearance did not differ significantly between 0-2 and 0-4 hours, but after 4 hours the clearance is significantly less ($p<0.001$). The mean total body clearances were 40, 37, 32, and 30 ml/min/1.73 m^2 for 0-2, 0-4, 0-6 and 0-8 hours respectively.

Correlation between total body clearance and renal clearance of inulin following a single injection.

A plot of the total body clearance of inulin against the renal clearance is shown in Fig 2.3.9. This includes data from both the 23 healthy subjects (0-2 hour data), and the 8 patients with renal impairment (0-4 hour data). The total body clearance significantly overestimated the renal clearance of inulin ($p< 0.005$) by a mean of 6% (87 v 82 ml/min/1.73 m^2). There was a significant correlation

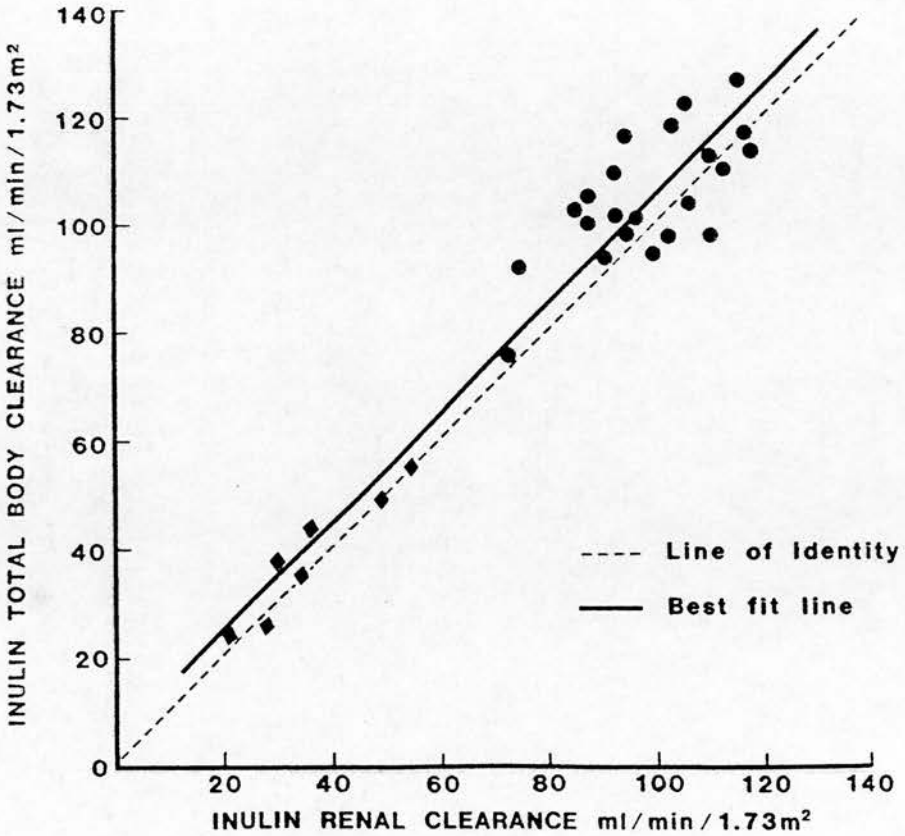
TABLE 2.3.4

Total body clearance of inulin (ml/min/1.73 m^2) after a single intravenous injection of inulin in 23 healthy male subjects.

	COLLECTION PERIOD (hours)		
SUBJECT	0-2	0-4	0-6
PF	123	123	104
GS	105	84	54
JN	76	63	54
DM	104	62	76
MK	109	95	99
CP	94	79	79
BH	92	80	38
BS	101	92	45
PL	114	85	93
TM	112	107	99
GW	101	82	77
AT	127	113	113
EC	95	92	82
WW	101	85	82
AB	98	97	82
RJ	118	99	88
AD	117	109	97
JA	99	93	92
RF	113	103	93
PD	98	89	78
MS	103	88	81
SA	117	108	91
JG	98	100	96
MEAN	105	93	82
±SD	12	15	19

Fig 2.3.9

Relationship between inulin total body clearance and the corresponding inulin clearance in 23 healthy male subjects (●) and 8 patients with renal impairment (◆).



between the total body clearance, and single injection renal clearance ($r= 0.97$, $p< 0.001$, Fig 2.3.9).

Study three.

The individual total body clearances are given in Table 2.3.3B. The mean total body clearances in the "step up" study were 96, 101, and 121 ml/min/1.73 m^2, for low mid and high plasma concentrations respectively. This increase was significant over time and increasing plasma concentration ($p< 0.01$). Both low and mid total body clearances, were significantly lower than that for the high plasma concentration ($p< 0.005$). The corresponding mean values for the renal clearance of inulin were 90, 97, and 114 ml/min/1.73 m^2, and the ratios of the mean total body clearances to the mean renal clearances were close to unity (1.07, 1.04 and 1.06 respectively).

The total body clearance of inulin could not be calculated for the "step down" studies as steady state plasma concentrations were not achieved.

Creatinine clearance

Study one.

The individual creatinine clearances are given in Table 2.3.5. The mean creatinine clearances were 130, 113, 117, 120, and 107 ml/min/1.73 m^2, for the collection periods 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 hours respectively (Fig 2.3.4). There was a significant change in creatinine clearance over time ($p< 0.05$), with the 0-1 hour clearance being significantly greater than all other collection periods, ($p<0.01$) except 4-6 hours. No significant differences were found between the other collection periods.

Study two.

The individual creatinine clearances are given in Table 2.3.6A. The mean creatinine clearance showed a small, but progressive decline up to four hours (Fig

TABLE 2.3.5

Renal clearance of creatinine (ml/min/1.73 m^2) during the 8 hours after a single intravenous injection of inulin in 23 healthy male subjects.

	COLLECTION PERIOD (hours)					
SUBJECT	0-1	1-2	2-3	3-4	4-6	6-8
PF	209	107	128	119	108	100
GS	120	113	106	108	109	102
JN	117	112	112	111	119	118
DM	129	124	130	125	131	114
MK	*	125	127	102	113	61
CP	131	109	111	99	116	93
BH	146	119	103	108	98	110
BS	136	129	115	115	244	121
PL	173	123	125	123	105	126
TM	119	123	115	114	123	129
GW	105	79	114	113	109	102
AT	111	118	104	113	129	123
EC	124	111	111	115	108	49
WW	98	95	102	96	95	105
AB	126	113	118	125	123	136
RJ	109	108	109	110	129	113
AD	124	120	122	119	115	106
JA	122	112	113	105	110	126
RF	217	131	128	133	123	119
PD	99	82	125	106	115	33
MS	118	108	107	116	106	119
SA	124	114	139	120	109	122
JG	110	131	128	123	128	128
MEAN	130	113	117	114	120	107
±SD	31	14	10	9	29	26

* clearance missing due to inaccuratly timed collection period

TABLE 2.3.6A

Renal clearance of creatinine (ml/min/1.73 m^2) following an intravenous bolus of inulin in 8 renal impaired patients

	COLLECTION PERIOD (hours)					
SUBJECTS	0-1	1-2	2-3	3-4	4-6	6-8
JB	36	33	34	32	31	33
EC	33	33	30	31	26	34
AD	38	39	40	38	39	43
EH	38	38	39	38	35	37
WA	61	54	63	57	61	59
JH	65	66	62	61	63	61
CP	62	35	53	33	61	58
EB	46	48	33	33	47	47
MEAN	48	45	44	40	45	46
±SD	13	12	13	12	15	12

TABLE 2.3.6B

Urine flow rate (ml/min) following an intravenous bolus of inulin in 8 renal impaired patients

	COLLECTION PERIOD (hours)					
SUBJECTS	0-1	1-2	2-3	3-4	4-6	6-8
JB	3.9	3.9	4.7	4.5	3.0	2.8
EC	2.5	4.2	4.2	4.5	2.9	3.4
AD	6.4	5.7	5.0	4.2	3.0	3.4
EH	5.6	5.7	6.2	5.3	2.8	3.0
WA	6.3	5.7	6.8	5.8	3.8	3.1
JH	7.4	5.7	5.8	5.0	2.2	2.3
CP	6.2	6.3	6.1	3.9	4.6	6.2
EB	6.9	6.9	4.4	4.1	4.5	4.5
MEAN	5.6	5.5	5.4	4.7	3.4	3.6
±SD	1.6	1.0	1.0	0.7	0.9	1.2

2.3.6). The mean clearances were 48, 45, 44, 40, 45 and 46 ml/min/1.73 m^2 for 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 hours respectively. There was no significant change over time.

Study three.

The individual creatinine clearances are given in Table 2.3.7A. The mean creatinine clearances were similar in all collection periods in the "step up" and "step down" studies (Fig 2.3.7) (136, 141, and 135 ml/min/1.73 $m^{2)}$ for high, mid and low periods in the "step up" study, and 137, 147, and 135 ml/min/1.73 m^2 for the corresponding period during the "step down" study. There was no significant change in creatinine clearance over time on either day.

The relationship between the renal clearance of inulin and creatinine and urine flow rate

Study one.

The individual urine flow rates for each period are shown in Table 2.3.8. The mean urine flow rate fell, progressively, from 7.1 to 2.7 ml/min from 0-1 to 6-8 hours. This was significant over 8 hours ($p< 0.01$). There were no significant correlations between the renal clearances of inulin or creatinine and the corresponding urine flow rates except, in the last collection period ($r= 0.66$, $p< 0.001$ and $r= 0.45$, $p<0.05$ for inulin and creatinine respectively, Figs 2.3.10 A & B).

Study two.

The individual urine flow rates for each period are given in Table 2.3.6B. The mean urine flow rate fell from 5.6 to 3.6 ml/min from 0-1 to 6-8 hours. This fall was insignificant over 4 hours, but was significant over 8 hours ($p< 0.01$). A significant correlation was found between the urine flow rate, and the corresponding renal clearance of inulin for the collection periods 0-1 ($r =$

TABLE 2.3.7A

Renal clearance of creatinine (ml/min/1.73 m^2) during incremental changes in the inulin dose rate in 8 healthy male subjects

	COLLECTION PERIOD					
	STEP UP			STEP DOWN		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
JN	119	128	117	117	120	117
GS	133	132	131	149	141	131
SB	135	128	130	141	167	180
AD	126	163	141	133	143	135
BB	124	128	126	130	141	132
WW	155	162	148	162	199	141
SM	169	158	150	126	131	134
BW	120	129	142	121	133	129
MEAN	135	141	136	135	147	137
±SD	18	17	11	15	25	19

TABLE 2.3.7B

Urine flow rate (ml/min) during incremental changes in the inulin dose rate in 8 healthy male subjects

	COLLECTION PERIOD					
	STEP UP			STEP DOWN		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
JN	7.2	7.9	8.8	9.6	5.9	10.1
GS	8.1	8.7	8.5	8.1	9.5	7.5
SB	9.6	4.4	9.6	4.1	4.8	7.6
AD	7.6	9.3	6.7	8.3	8.5	8.6
BB	11.0	9.5	10.0	9.4	7.0	8.5
WW	11.7	8.9	8.0	12.3	12.3	12.7
SM	7.8	7.0	6.8	8.6	6.9	8.4
BW	6.4	5.0	8.9	7.5	6.1	6.1
MEAN	8.7	7.6	8.4	8.5	7.6	8.7
±SD	1.9	2.0	1.2	2.3	2.4	2.0

TABLE 2.3.8

Urine flow rate (ml/min) during the 8 hours after a single intravenous injection of inulin in 23 healthy male subjects.

	COLLECTION PERIOD (hours)					
SUBJECT	0-1	1-2	2-3	3-4	4-6	6-8
PF	12.7	5.5	3.7	2.4	5.3	3.5
GS	5.5	7.4	5.8	5.1	1.5	3.0
JN	6.0	7.3	6.8	7.2	3.2	5.6
DM	1.6	5.7	4.6	6.0	5.2	1.4
MK	*	7.1	5.7	3.4	2.9	2.0
CP	0.6	1.7	8.3	6.1	7.5	1.1
BH	7.5	7.2	6.0	5.7	0.9	1.6
BS	10.7	6.3	4.3	5.0	1.7	1.4
PL	13.1	6.9	6.4	6.2	4.0	2.6
TM	8.6	5.1	5.8	4.3	4.2	2.0
GW	1.5	9.8	6.2	5.7	5.9	2.8
AT	6.0	4.8	7.4	6.3	3.0	6.4
EC	8.4	5.8	5.4	5.4	1.2	0.3
WW	8.4	4.3	5.8	3.8	3.0	4.0
AB	10.9	5.5	5.3	5.8	2.0	2.7
RJ	7.7	6.8	2.6	5.2	3.2	1.3
AD	7.5	4.8	4.9	6.3	5.0	2.0
JA	6.7	4.6	5.9	3.6	4.8	4.4
RF	4.3	8.5	3.6	7.3	2.7	1.3
PD	8.4	4.8	4.5	3.6	2.3	1.0
MS	7.9	5.6	5.1	5.0	3.5	4.7
SA	9.6	5.2	5.9	2.5	3.6	2.8
JG	9.3	7.0	4.9	5.5	4.6	3.3
MEAN	7.4	6.0	5.4	5.1	3.5	2.7
±SD	3.3	1.6	1.3	1.4	1.6	1.6

* clearance missing due to inaccuratly timed collection period

Fig 2.3.10A

Relationship between the renal clearance of inulin and urine flow rate (6-8 h), after a single injection of inulin in 23 healthy male subjects.

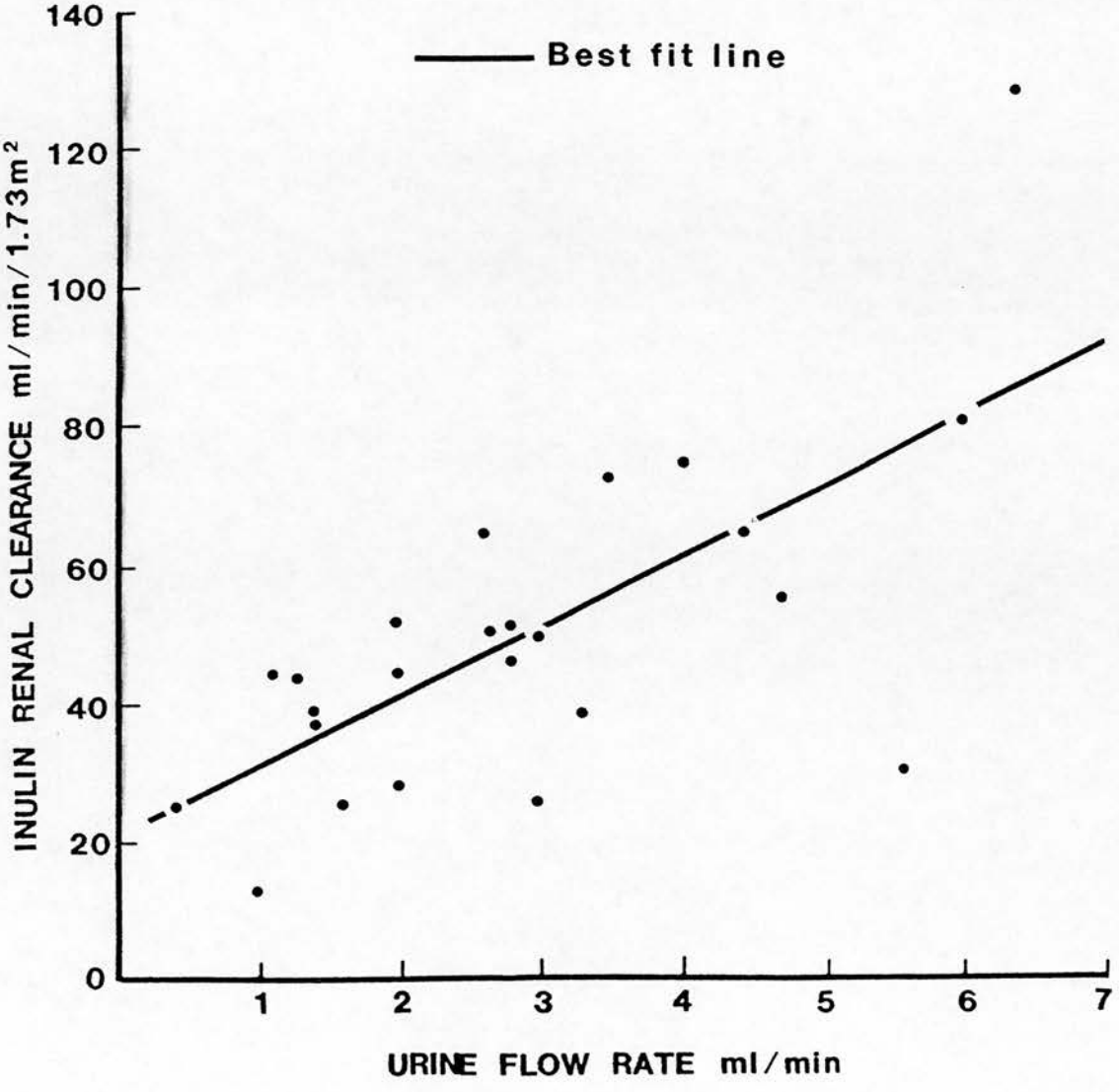
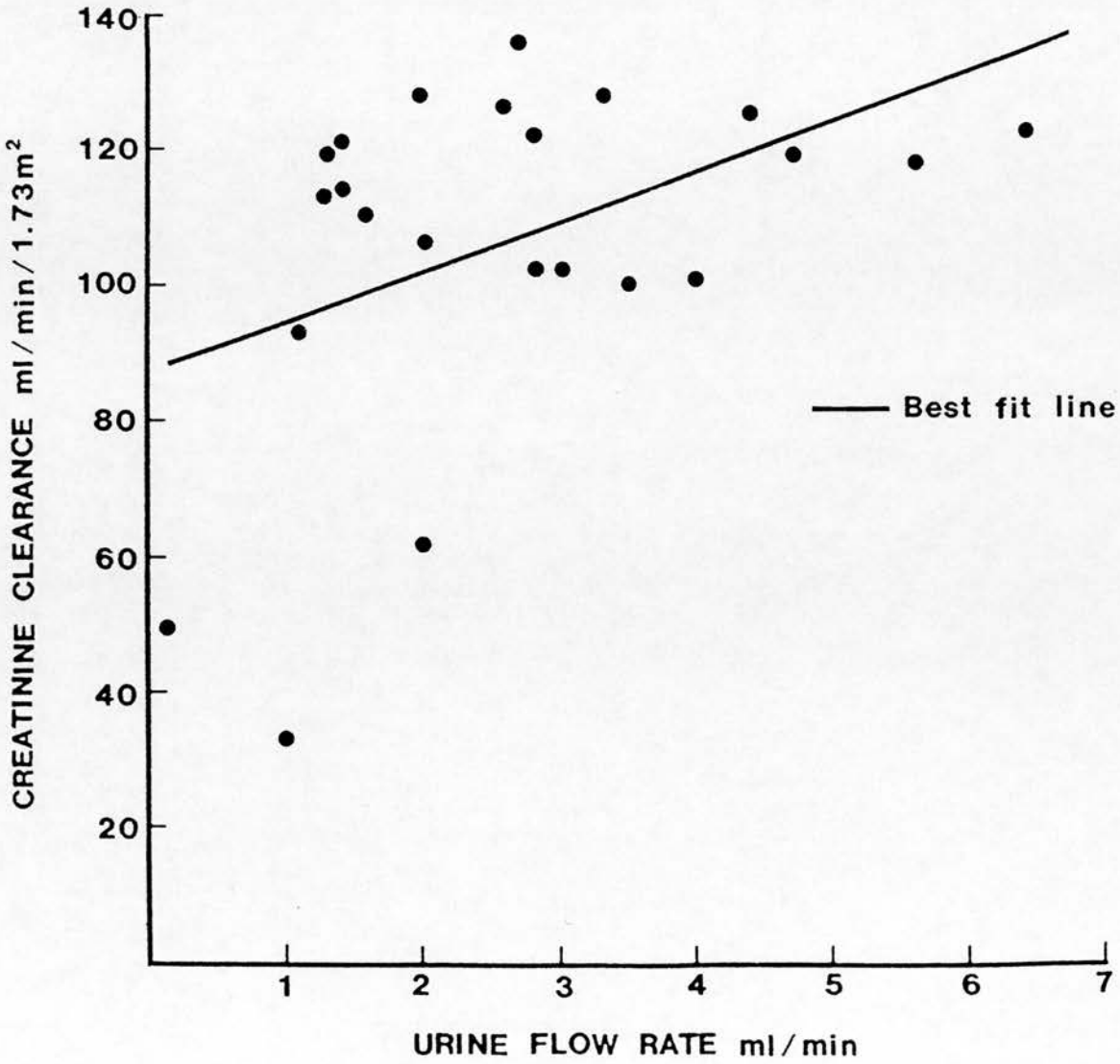


Fig 2.3.10B

Relationship between the renal clearance of creatinine and urine flow rate (6-8 h), after a single injection of inulin in 23 healthy male subjects.



0.84, $p<0.01$), 2-3 and 3-4 h ($r= 0.75$ and $r= 0.72$, $p< 0.05$, Figs 2.3.11 A, B, & C), and for the creatinine clearance 2-3 h ($r= 0.82$, $p< 0.01$ Fig 2.3.11 D). There was no such correlation for any other periods.

Study three.

The individual urine flow rates for each period are shown in Table 2.3.7B. The mean urine flow rate for each period during the two study days, were similar. No correlation between the inulin or creatinine renal clearance and the corresponding urine flow rate, were found.

Urinary recovery of inulin

Study one.

Over 24 hours the mean percentage recovery of the dose administered in the urine was 102 $\pm$ 7 %. Of this, 50 % is recovered in the first hour, and 97 % is excreted over eight hours. The individual recoveries are given in Table 2.3.9.

Study two.

Over the eight hour study duration, the mean percentage recovery of the dose administered in the urine was 78 %. Of this, 50 % was excreted between 2 and 6 hours depending on the degree of renal failure. No 8-24 hour collection was made. The individual recoveries are given in Table 2.3.10A.

Study three.

The mean urinary recovery of inulin in both studies are given in Table 2.3.10B. For the "step up" study, the percentage recovery of inulin relative to the amount infused, was similar over the three periods (96 $\pm$ 7, 98 $\pm$ 7 and 98 $\pm$ 8 % respectively). For the "step down" study, the amount recovered exceeded the amount infused for the

Fig 2.3.11

Relationship between the renal clearance of inulin (0-1 h (A), 1-2 h (B), and 3-4 h (C), and creatinine (2-3 h (D) with urine flow rate, after a single injection of inulin in 8 patients with renal impairment.

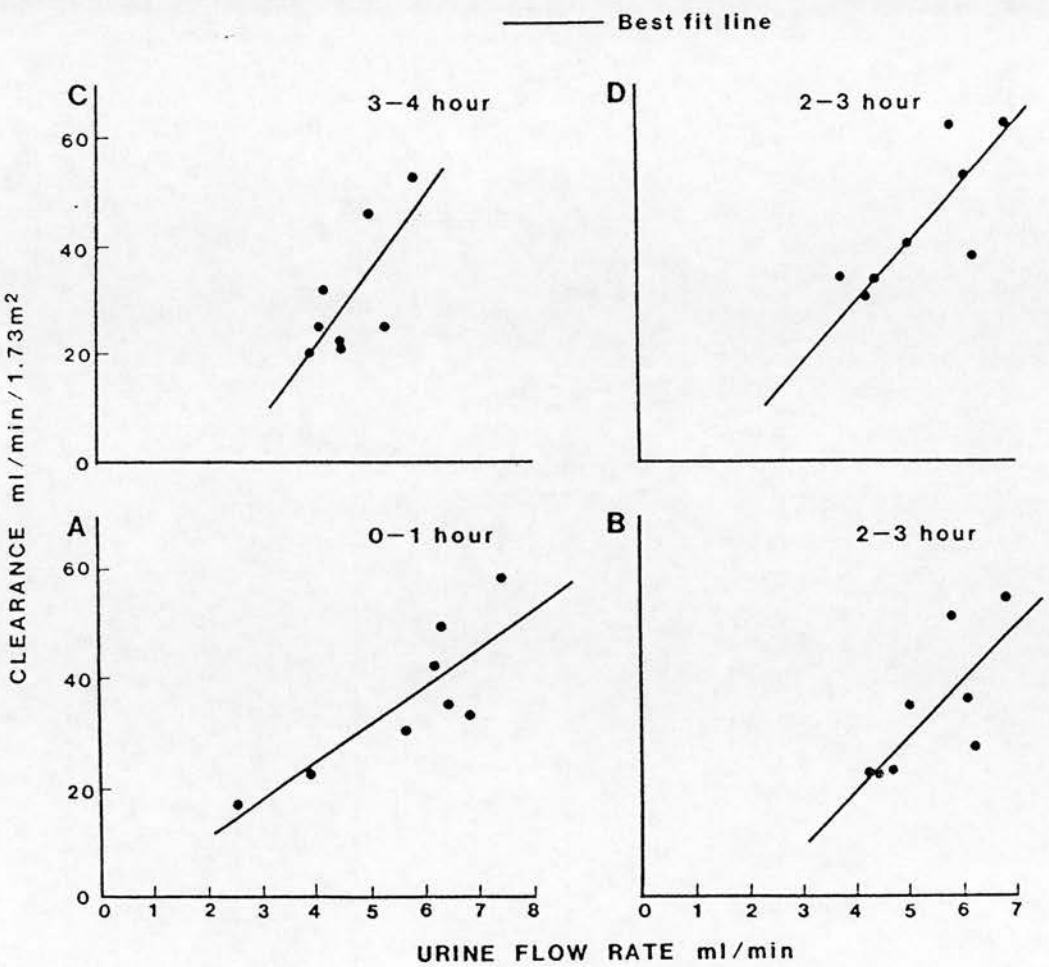


TABLE 2.3.9

Percentage urinary recovery of inulin after rapid intravenous administration of inulin in 23 healthy male subjects

	COLLECTION PERIOD (hours)							
SUBJECT	0-1	1-2	2-3	3-4	4-6	6-8	8-24	TOTAL (0-24)
PF	55	15	8	5	5	2	6	96
GS	50	19	9	6	6	3	7	100
JN	43	18	12	8	8	5	8	102
DM	53	20	10	7	6	3	8	107
MK	40	19	11	5	5	1	5	86
CP	50	20	9	7	8	3	5	102
BH	46	19	9	6	6	3	5	94
BS	49	19	10	6	7	3	9	103
PL	59	19	11	7	7	4	7	114
TM	56	20	10	6	7	3	8	110
GW	46	20	11	7	8	4	3	99
AT	52	23	10	5	5	3	10	108
EC	60	20	11	7	7	1	9	115
WW	55	18	11	6	6	4	5	105
AB	63	18	10	6	6	2	1	106
RJ	52	16	8	7	7	3	9	102
AD	65	18	10	5	4	2	5	109
JA	54	16	9	5	6	3	4	97
RF	57	18	9	6	6	3	5	104
PD	50	26	13	6	2	1	6	104
MS	52	16	9	6	5	4	6	98
SA	48	16	11	5	5	3	6	94
JG	57	23	9	6	6	2	4	107
MEAN	53	19	10	6	6	3	6	103
±SD	6	3	1	1	1	1	2	7

TABLE 2.3.10A

Urinary recovery of inulin as the percentage of dose administered in 8 patients with renal impairment over 8 hours

	COLLECTION PERIOD (hours)						
SUBJECTS	0-1	1-2	2-3	3-4	4-6	6-8	TOTAL(0-8)
JB	18	13	10	8	12	9	68
EC	12	13	9	8	11	11	62
AD	27	16	12	8	13	9	85
EH	24	14	11	9	12	10	79
WA	35	16	12	9	11	8	91
JH	41	18	11	7	9	6	91
CP	29	16	7	4	12	7	75
EB	25	15	7	6	12	8	72
MEAN	26	15	10	7	11	8	78
±SD	9	2	2	2	1	2	11

TABLE 2.3.10B

Percentage urinary recovery of inulin relative to dose administered in 8 males with normal renal function during incremental infusions

	COLLECTION PERIOD					
	STEP UP			STEP DOWN		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
JN	89	99	92	110	178	196
GS	86	83	92	106	160	189
SB	84	89	83	102	192	228
AD	95	99	94	115	175	170
BB	108	98	104	112	186	174
WW	99	101	91	120	253	197
SM	100	100	100	101	149	172
BW	97	103	113	136	200	222
MEAN	95	97	96	113	187	194
±SD	8	7	9	11	32	22

first period, and as the infusion rate was decreased, the discrepancy increased. The mean recoveries were 114 ± 11, 186 ± 34 and 194 ± 22 %, for high, mid and low periods respectively.

The relationship between the urinary excretion rate and inulin plasma concentration.

If the renal clearance of a substance is independent of its plasma concentration, a plot of urinary excretion rate against plasma concentration, should give a straight line passing through the origin, and the slope should be equal to the renal clearance (Tucker, 1981).

Study one.

The log-log plot of the mean urinary excretion rate of inulin against the mean plasma concentration of inulin, is shown in Fig 2.3.12. It has been assumed that the first collection period gives an accurate estimate of the GFR and therefore a straight line relationship should exist between this point, and the origin, if the renal clearance of inulin is independent of its plasma concentration. This graph shows clearly that, at low plasma concentrations the points deviate from linearity. In Fig 2.3.13, the last four points are plotted on linear graph paper, to show the deviation from the origin. If the best straight line is drawn through these points, the line cuts the x axis at 16 mg/l. In other words, at this level, the renal clearance of inulin falls to zero. The solid line in this graph represents the straightline relationship between the first collection period, and the origin.

Study two.

Similar plots of the urinary excretion rate against plasma concentration in these patients, as in subjects with normal renal function, are shown in Fig 2.3.12 & Fig 2.3.13. The slope of the line joining through the first

Fig 2.3.12

Relationship between the mean urinary excretion rate of inulin and the mean plasma concentration of inulin in 23 healthy males and 8 patients with renal impairment, following a single injection. The lines represent the straight line relationship between the highest clearance periods and the origin for subjects with normal (———) and impaired renal function (------). Bars = ± SD.

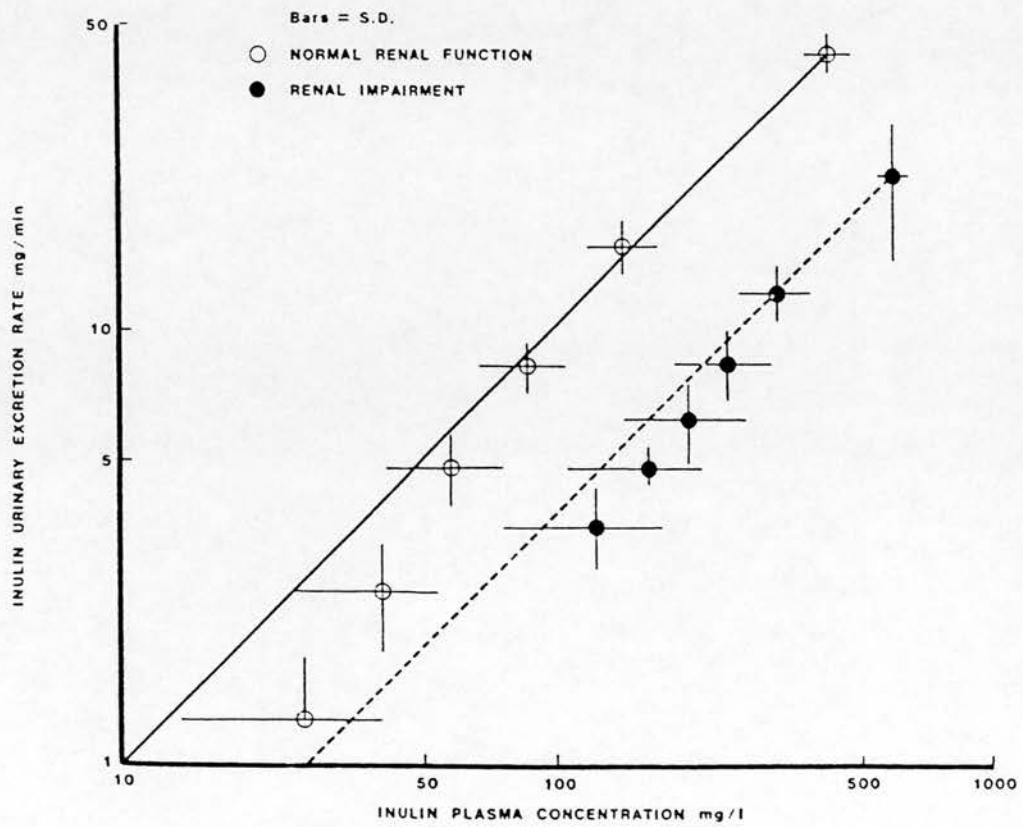
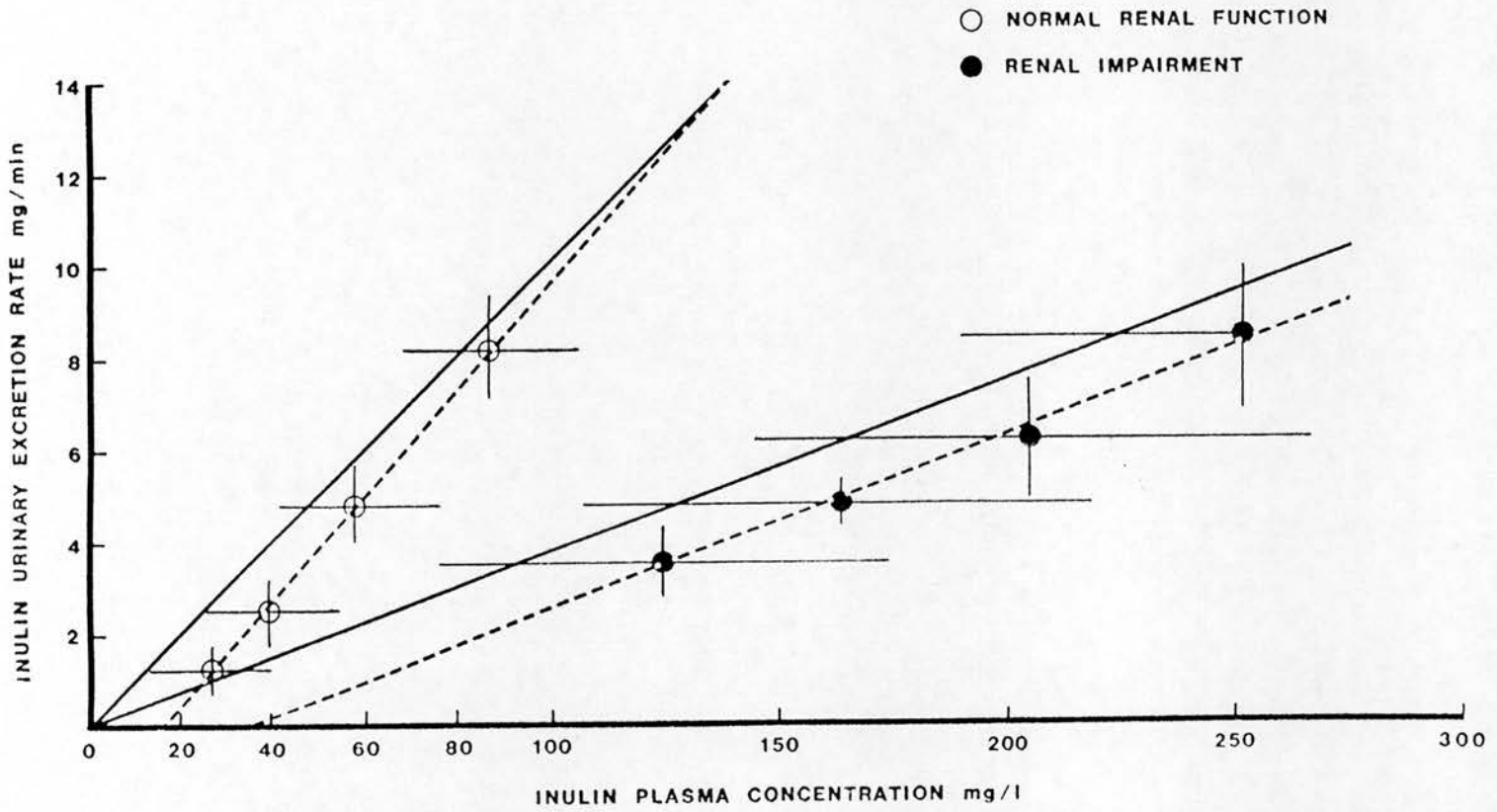


Fig 2.3.13
Relationship between the mean urinary excretion rate of inulin and the mean plasma concentration of inulin in 23 healthy males and 8 patients with renal impairment, following a single injection (last four clearance periods only). The solid line represents the straight line relationship between the highest clearance period and the origin. The dashed line represents the best straight line through the points. Bars = ± SD.



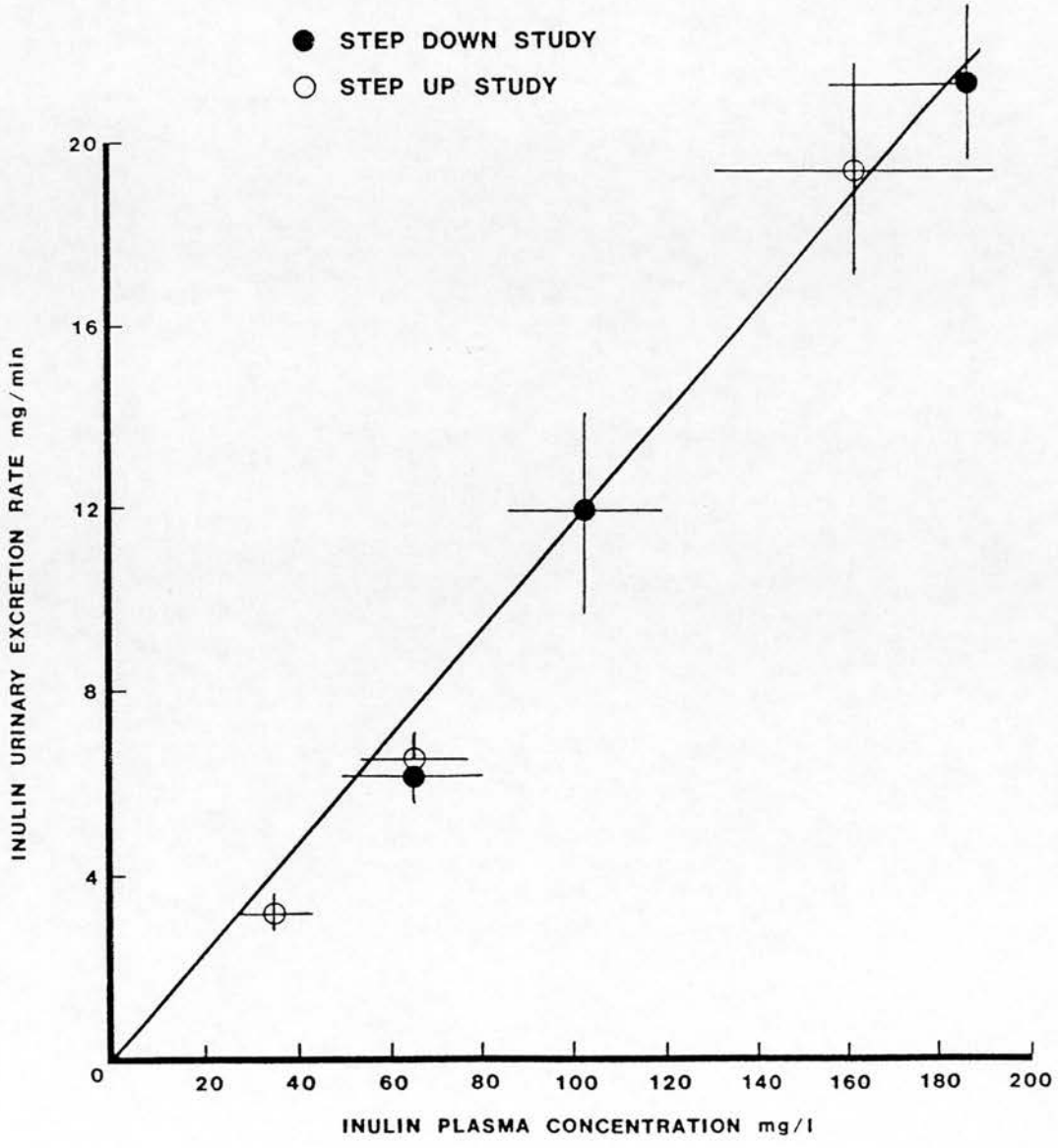
period and the origin, is shallower as would be expected in subjects with reduced renal function. On drawing the best straight line through the last four points, it is found that the line cuts the x axis at 35 mg/l.

Study three.

The plot of mean urinary excretion rate against plasma concentration for both the "step up" and "down" studies are shown in Fig 2.3.14. If a line is drawn through the points for the clearance periods when the plasma concentration was highest and the origin, it can be seen that there is a small deviation from this line for the low and mid periods during the "step up", and only for the low on the "step down". The divergence from the origin is not as great as that seen after a single injection.

Fig 2.3.14

Relationship between the mean urinary excretion rate of inulin and the plasma concentration of inulin during step up and step down constant infusion studies, in 8 healthy males. The solid line represents the straight line relationship between the highest clearance periods and the origin. Bars = ± SD.



DISCUSSION

Inulin clearance has been used for over 50 years as the "gold standard" for measuring glomerular filtration rate (Schuster & Seldin, 1985). Inulin best fulfils the criteria for measurement of the GFR, as its urinary excretion rate is thought to be proportional to the plasma concentration, over a wide range 50 to 4000 mg/l, and the clearance of substances, such as PAH and glucose, after inhibition of tubular secretion and reabsorption (by phlorizin and saturation) respectively, are equal to that of inulin. Inulin is thought to be freely filtered at the glomerulus, and to be neither secreted or reabsorbed by the kidney tubules, synthesised or metabolised by the body (Smith, 1951; Pitts, 1968). The inulin clearance is therefore considered to be independent of plasma concentration and urine flow rate (Schuster & Seldin, 1985).

However, under the conditions of the present study, both total body and renal clearances of inulin fell progressively after a single injection with the passage of time, declining plasma concentrations and falling urine flow rates. The same effect were seen in subjects with normal renal function and patients with impaired renal function. This fall was most dramatic in healthy subjects with normal renal function, after two hours. There was no change in the simultaneously measured creatinine clearance. In patients with renal impairment, a progressive fall in inulin clearance was seen after two hours, but this did not become statistically significant until after four hours. Despite the apparent decline in inulin clearance with time and decreasing plasma concentrations, the single injection method gives an adequate estimate of the GFR, if sampling is continued for 2 hours in healthy subjects, and 4 hours in patients with moderate impairment of renal function. There was also a good correlation between the renal

clearance and total body clearance of inulin (r= 0.96), although the latter overestimated the former by about 6 %.

Similarly, a concentration dependant effect on the renal and total body clearances of inulin were seen at different steady state inulin plasma concentrations, produced by, incremental stepping up or down the infusion rate, in subjects with normal renal function. Steady state plasma concentrations were reached in the "step up", but not "step down" study. In the latter study, this was due to excessive input of inulin at a time when extracellular concentrations were higher than the plasma concentrations. This is reflected in the greater urinary recovery than the infused dose, and therefore, the total body clearance of inulin could not be calculated in the step down study. No change in creatinine clearances were seen as the inulin infusion rates were stepped up or down.

The inulin clearance clearly changed significantly in all three studies, and in each of these, three associated variables:- time, changing plasma concentration, and urine flow rate could have been involved. These variables are now considered in turn.

1) The effect of time on the clearance of inulin.

Following a single injection of inulin, the clearance of inulin fell significantly with time after 2 hours in patients with normal renal function, and after 4 hours in patients with renal impairment. During the constant infusion step up studies, the clearance rose significantly with time, whilst during the step down, the clearance fell with time. These studies were carried out at the same time of day, and the mean clearances of inulin at the high plasma concentrations on both step up and step down study days were similar, even though in one study, the clearance was calculated in the 1½ to 2½ hour period and the other, from 5½ to 6½ hours. Smith

(1951), reported a systematic drift downwards in the filtration rate during long constant infusion studies, but gave no figures. In the constant infusion study reported in section one there was no change in inulin renal clearance over a four hour infusion period. Yet four hours after a single injection of inulin, the clearance had dropped significantly, in subjects with normal renal function. The glomerular filtration rate is subject to diurnal changes of ± 10 %, with the lowest rate at 4.00 am, and highest at 2.30 pm (Wesson & Lauler, 1961). Altogether these observations suggest that the inulin clearance is not dependant on time, although a small diurnal effect is possible.

Selective filtration of lower molecular weight fractions of inulin with time, has previously been suggested as one possible reason for the decline in inulin clearance, following a single injection (Ferguson et al, 1950; Barnard et al, 1955). This is considered below:

Selective filtration

Inulin is a complex mixture of polymers, consisting of some 30 fructofuranose subunits with an average molecular weight of 5200. However, it is not homogeneous, and it contains a wide range of polymers with molecular weights up to 15,000. Different batches and sources of inulin have different molecular weight ranges (Bassir, 1956). "Inutest" is claimed to be more homogeneous, but has a molecular weight range of 800 - 16,000 (Nitsch et al, 1979). Selective filtration of the lower molecular weight polymers of inulin, would result in retention of the higher weight species, which might be filtered less effectively. Such an effect, would produce a decreasing clearance with time (Barnard et al, 1955). Time is unlikely to be a crucial factor as described above. There is evidence that inulin is not freely filtered at the glomerulus in rats (Berglund,

1965), but this has not been confirmed by micropuncture studies (Gutman et al, 1965; Harris et al, 1974). The molecular weight patterns of inulin in plasma and eluting in urine, were similar in normal subjects, patients with renal impairment and pre-term babies (Mogensen, 1968; Coulthard & Ruddock, 1983). Thus, inulin appears to be freely filtered in man, and has been used for the estimation of glomerular pore size (Brenner et al, 1978)

2) The effect of urine flow rate on the clearance of inulin

The results of the present study show that the effects of urine flow rate on the inulin clearance in subjects with normal renal function, is limited. Following a single injection, the inulin clearance falls progressively, and so does the urine flow rate, but there was no significant correlation until the last collection period. The relationship between creatinine clearance and urine flow rate was similar, but there was no fall in the creatinine clearance until the last collection period, which suggests incomplete bladder emptying. There was also no correlation between urine flow rate and inulin clearance in the constant infusion studies, despite significant changes in the inulin clearance. In the patients with renal impairment, there was no significant change in urine flow rate over the first 4 hours, but in 3 of the four collection periods there were significant correlations with the inulin clearance. However, the urine flow rate and inulin clearance both fell after 4 hours, but were not correlated. The urine flow rate was high (5 ml/min), and as there was no correlation between the creatinine clearance and urine flow rate for all but one of the periods, incomplete bladder emptying seems unlikely. The reason for these sporadic correlations is not known.

Accurate measurement of urine flow rate depends upon

correct timing of the collection periods and complete bladder emptying. In this study, care was taken to minimalize errors by hydration and long urine collection periods. In any event, normal variation in urine flow rate is due to changes in water reabsorption, rather than in filtration rate (Pitts, 1968). If there is no tubular reabsorption of inulin (Gutman et al, 1965), there should be no correlation between the inulin clearance and urine flow rate. The renal clearance of inulin is said to be independent of urine flow rate over the range of 1 to 14 ml/min (Chasis & Smith, 1938), but the filtration rate may fall with dehydration and increase with hydration. The latter effect is thought to be due to an increase in extracellular space (Smith, 1951).

3) The effect of plasma concentration on inulin clearance

The plasma concentration of inulin declined during the single injection and "step down" studies, and rose in the "step up" study. These changes were consistently mirrored by the clearance of inulin. At first sight, the changes in the inulin renal clearance during the constant infusion "step up" and "down" studies, do not seem as dramatic as those following a single injection of inulin over a similar range of plasma concentrations. However, the rise in clearance during the step up study was about 27 %, with plasma concentrations increasing from 35 to 165 mg/l, and over a similar plasma concentration range after single injection, the fall in the inulin renal clearance was 38 %. The fall in clearance seen in the step down study was 16 % as plasma concentrations fell from 187 to 65 mg/l, and over a similar plasma concentration range, following single injection, the fall in renal clearance was the same. In patients with renal impairment, the change in renal clearance as plasma concentrations fell from 550 to 130 mg/l, was 19 %. The changes in total body clearance of

inulin over the same periods were 26, 22, and 25 % for the step up and single injection studies, in subjects with normal and impaired renal function respectively.

One possible reason for the difference in percentage change in renal clearance of inulin over similar plasma concentrations during the step up and single injection studies in subjects with normal renal function, may be arterio-venous differences in the latter, which should be minimal in constant infusion studies (Chiou & Lam, 1982). As inulin is cleared from the arterial side of the kidney but venous blood is sampled, there may be differences in the arterial and venous concentrations of inulin with continuously rising and falling plasma concentrations. After an intravenous bolus, the arterial concentrations of inulin are greater than venous concentrations, as inulin moves from the circulation into extracellular stores. When distribution is complete, inulin moves in the reverse direction, and the venous plasma concentrations are higher than arterial concentrations. This distribution effect occurs slowly, and over two hours, venous concentrations in one study were 7.4% higher than arterial concentrations (Brun et al, 1949). After two hours, this difference could be greater, and accentuate the fall in renal clearance after a single injection. Other investigators, have discounted arterial-venous differences as a cause for the fall in inulin clearance after a single injection (Ferguson et al, 1950; Laake, 1954), and the results of the present "step up" study, show a change in clearance in circumstances where there is no significant arterial-venous difference in concentrations. The percentage change in the total body clearances of inulin in the different studies were similar, and this is less likely to be influenced by arterial-venous differences as, after 2 hours the curve is extrapolated to infinity, and the pre- and post- distribution effects will more or

less cancel out (Nosslin, 1965).

In subjects with normal renal function, the renal clearance of inulin fell when plasma concentrations dropped below 100-120 mg/l. However, these studies provide no evidence that the renal clearance of inulin remains constant above this level, and the possibility of "supra normal" clearance at much higher plasma concentrations, cannot be excluded.

The renal clearance of inulin has previously been reported to be independent of plasma concentrations between 40 to 4000 mg/l, with both declining plasma concentrations, and at different steady state levels (Shannon & Smith, 1935; Miller et al, 1940; Kennedy & Kleh, 1953; Cole et al, 1972). On the other hand, others have reported a falling clearance of inulin after a single injection, and different clearances at different constant infusion levels (Josephson & Lindhal, 1943; Ferguson et al, 1950; Barnard et al, 1955; Laake, 1954). The reasons for these contradictory findings are obscure, but it is certain that the renal clearance of inulin is not as constant as claimed by many investigators.

The more dramatic decrease in inulin clearance observed in this study, compared to previous studies, could be explained by longer sampling periods, and the measurement of clearance at plasma concentrations lower than those studied by most other investigators (Shannon & Smith, 1935; Josephson & Lindhal, 1943; Ferguson et al, 1950; Laake, 1954; Cole et al, 1972). Miller et al, (1940), used the constant infusion technique in 3 subjects with normal renal function, and although there was a difference in mean clearance between low (below 100 mg/l), and high inulin plasma concentrations (above 100 mg/l), this was dismissed by the author. In another 3 healthy subjects using a single injection technique, they found a fall in clearance in one subject and a rise

with the other two, as the plasma concentrations fell. These results are difficult to interpret, because the kinetic analysis was inappropriate, and samples were inappropriatly timed. Ferguson et al, (1950), showed a fall in inulin clearance following single injection and constant infusion at different plasma levels, but these were higher than 100 mg/l. Similar findings following single injection were reported by Barnard et al, (1955). Again, the methods of analysis were inappropriate, and sampling was limited. Josephson & Lindhal (1943), reported a fall in inulin clearance in the third urine collection period after single injection, but no plasma concentration data was given, so no inference on the relationship between plasma concentration and renal clearance can be drawn. The findings of Laake (1954), were similar to those in the present study in healthy subjects, and the clearance seemed to fall after the plasma concentrations reached 100 mg/l. The author concludes that this is due to a decrease in extracellular fluid tonicity, and thus intrarenal pressure due to fluid loading. However Smith (1951), reports that the filtration rate increases with hydration. Kennedy & Kleh (1953), found no concentration dependant clearance of inulin during constant infusions, in which plasma concentrations of inulin were stepped up from 40 mg/l to 900 mg/l.

The fall in inulin clearance in the patients with renal impairment occurred at higher plasma concentrations than in subjects with normal renal function, but the actual change was much smaller. Similar findings were reported by Ferguson et al, (1950), and Laake, (1954). Thus, the critical plasma concentration threshold seems to depend on renal integrity and function. The simultaneous endogenous creatinine clearance was not measured by other investigators, and it is not known if the changes were due to natural

fluctuation in the GFR or incomplete bladder emptying. The consistency of the creatinine clearance in the present study, suggests that the fall in inulin clearance was not due to these factors. More recent studies of the total body clearance of inulin have correctly utilised two compartment or model independent kinetic analysis, but no decline in the renal or total body clearance of inulin, has been noted. This can probably be explained by the measurement of plasma concentrations above the critical level, or the use of patients with reduced renal function, where small changes in clearance may go unnoticed (Ladegaard-Pedersen, 1972; Broberger, 1973; Svenningsen, 1975; Fawer et al, 1979; Muller-Suur et al, 1983 ; Rehling et al, 1984).

The results of the present studies are supported, in part, by previous reports, and there seems no doubt that the clearance of inulin is dependant on its plasma concentration, below a certain level. The question arises to the mechanisms involved, and possibilities include increased extrarenal clearance of inulin, and tubular reabsorption.

Extrarenal clearance

The total body clearance of inulin exceeded the renal clearance by 6 %, in both single injection and constant infusion studies, in subjects with normal and impaired renal function. This excess represents either extrarenal clearance of inulin, or residual storage of inulin in the body. Inulin is not thought to be metabolised or cleared extrarenally, except possibly to an insignificant extent by biliary excretion (2%) (Smith, 1951). In addition, the urinary recovery of inulin following a single injection in subjects with normal renal function, was also complete, as found by others (Shannon & Smith, 1935). Therefore, any extrarenal clearance must be insignificant.

Tubular reabsorption of inulin

The possibility of tubular reabsorption of inulin at low plasma concentrations was raised by Ferguson et al, (1950), who found that a plot of urinary excretion rate against plasma concentration, gave a line which did not pass through the origin, but cut the y axis at a negative ordinate. A straight line passing through the origin would be expected if the urinary excretion rate is proportional to the plasma concentration, as is thought to be the case for inulin (Shannon & Smith, 1935). Similar analysis by Laake (1954), and Kennedy & Kleh (1953), gave different results. Either there was no deviation from the origin, or minor discrepancies were disregarded as insignificant. Laake (1954), suggested that the clearance falls as plasma concentrations decline below 100 mg/l, but no critical point could be determined. In the present study, the deviation from the line of identity is accentuated by the use of a log-log scale. The line crosses the x axis at a positive intercept (as shown on the linear graph) indicating that, at this plasma concentration the inulin clearance falls to zero. This negative deviation from the line of identity shows, that the rate of excretion of inulin lags behind the plasma concentration (Tucker, 1981). One explanation for this could be delay time; however, such a mechanism does not explain the change in inulin clearance during the "step up" and "step down" constant infusion. It is possible that delay time accentuates the fall following a single injection at low plasma concentrations, due to a diminishing urine flow rate. Another possible explanation is, that inulin is retained within the kidney as residual volume, as the urine flow rate diminishes, following a single injection. However, again the changes in both total body and renal clearances of inulin during the constant infusion study, in which urine flow rates were high and constant, suggests that

this is unlikely.

A more likely explanation for the concentration-dependent clearance of inulin is tubular reabsorption. If this occurs, reabsorption must either be saturable, or possibly a very small proportion is removed continuously, as the clearance of inulin is affected only at low plasma concentrations. The results are consistent with, but do not prove, reabsorption of inulin. The tubular reabsorption of inulin would also explain the consistent overestimation of the inulin renal clearance by the total body clearance of inulin in both single injection, and constant infusion studies, and also why the total body clearance of inulin rose, significantly, during the "step up" infusion study. Reabsorption should also be suspected if the renal clearance of a substance falls below the level of the glomerular filtration rate (Rowland & Tozer, 1980). The evidence against reabsorption of inulin comes mainly from micropuncture studies in rats (Gutman et al, 1965; Harris et al, 1974). These showed little (0.6-2.4 %) or no reabsorption as assessed by excretion via the contralateral kidney, and quantitative recovery of the amount injected. However, renal uptake of inulin occurs in animals (Balint & Forgacs, 1958; Gayer et al, 1961; Barber & Bourne, 1971).

The mechanism of reabsorption is not easily explained as, the molecular size of inulin excludes diffusion through tubular cell membranes, and it is not thought to be actively reabsorbed. In the rat, inulin does not diffuse through the plasma membrane of hepatocytes but, it undergoes endocytosis. Of this amount, 80 % was returned to the plasma, 18% was retained intracellularly, and 2 % was excreted in the bile (Scharschmidt et al, 1986). Any suggestion that renal tubular cells transport inulin in a similar way would be pure speculation, however, the ability to

internalize extracellular material appears to be a property shared by most cells (Scharschmidt et al, 1986). It has been reported that after administration of carbohydrates in large doses, vesiculation and swelling of the proximal tubule epithelium occurs (Maunsbach, 1973) and this has been described for inulin in rabbits (Simon et al, 1964). Although mechanisms cannot be identified, saturable tubular reabsorption of inulin cannot be excluded, and it is probably the most plausible explanation for the concentration-dependant decline in inulin clearance.

SUMMARY

The total body and renal clearances of inulin, change significantly with changes in plasma concentration, time and urine flow rate following a single injection in subjects with normal renal function, and patients with renal impairment. The clearance of inulin also changes over time and plasma concentration during constant infusion at different plasma levels. The creatinine renal clearance remained constant throughout each study, and therefore changes in inulin clearance were not due to changes in GFR. The passage of time and changes in urine flow rate are unlikely to account for all of the changes in the clearance of inulin. The fall in inulin clearance, seems to be plasma concentration dependant below a critical point. In subjects with normal renal function, this critical level was about 100 mg/l, but in patients with impaired renal function, it was much higher. The inulin clearance may fall at low plasma concentrations because the urinary excretion of inulin lags behind the plasma concentration, with increasing retention or reabsorption by the kidney. The higher total body than renal clearance of inulin could also conceivably be explained by reabsorption in the kidney. The mechanisms involved cannot be deduced from the present data, but the kinetics of the concentration-

dependent clearance of inulin are consistent with saturable tubular reabsorption. A reassessment of the role of inulin in renal function studies, is required.

SECTION FOUR

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

A single injection method for measuring the clearance of inulin was investigated and validated against the standard constant infusion method.

The results obtained and conclusions drawn are summarised below.

1) Over the first two hours following single intravenous administration of inulin (70 mg/Kg over 5 minutes in 10 healthy males), both the total body and renal clearances of inulin were similar (101 and 92 ml/min/1.73 m^2 respectively). They agreed well with the renal clearance of inulin during constant infusion (88 ml/min/1/73 m^2) in the same subjects.

2) With the single injection method, the total body and renal clearances of inulin decline progressively, and significantly after two hours, (a mean fall of 48 % over eight hours in the case of the renal clearance) and the correlation and similarity between the single injection and constant infusion methods, is lost.

3) The creatinine clearance does not show a similar fall, and a real alteration in GFR is therefore unlikely.

4) This depression in inulin clearance was investigated further following a single intravenous injection in 23 males with normal, and 8 patients with impaired renal function. The clearance of inulin was also investigated at different steady state plasma concentrations in 8 normal subjects by the use of "step up" and "step down" constant infusions.

5) In all three studies, there were significant changes in the clearance of inulin. This could have been

related to three variables, time, urine flow rate and plasma concentration.

6) The changes in inulin clearance are unlikely to be due to the effects of time, urine flow rate or arterial-venous differences, as significant changes were found in the "step up" and "step down" constant infusion studies, where these factors were reduced to a minimum.

7) The results of the present studies show that the inulin clearance depends on the plasma concentration below a certain level. This critical level is about 100 mg/l in normal healthy subjects, but is higher in patients with renal impairment.

8) Three significant factors are relevant to the mechanism behind the fall in clearance.

A) The inulin clearance falls to well below the glomerular filtration rate (creatinine clearance).

B) The total body clearance of inulin consistently overestimates its renal clearance, and the total body clearance also falls as the renal clearance declines. As the recovery of inulin in the urine is quantitative, major extrarenal clearance is unlikely.

C) The plot of inulin urinary excretion rate against plasma concentration indicates that, the urinary excretion rate of inulin lags behind the plasma concentration, as concentrations fall below 100 mg/l. Therefore, the fall could conceivably be due to relative retention of inulin in the urinary tract. This could occur by, 1) delay time error, 2) incomplete bladder emptying or 3) tubular reabsorption of inulin.

The first two factors do not adequately explain the results. However, the progressive and significant decline in inulin clearance below the GFR as the plasma

concentration declines, and the higher total body clearance than renal clearance of inulin, would be consistent with tubular reabsorption of inulin, which only becomes significant at low plasma concentrations. Saturable tubular reabsorption is the most plausible explanation for the observed concentration-dependent decline in inulin clearance.

9) The clearance of inulin following single intravenous administration over the first two hours, is similar to that found during the standard constant infusion method. Over this period, the single injection method was reproducible on repeated estimations, and the total body clearance of inulin varied less than the renal clearance of inulin as determined by single injection or constant infusion methods.

10) The single injection of inulin for measuring GFR is accurate and reproducible. It is also quick, easy and causes minimum discomfort to the patient, while the risks inherent with the use of radioactive compounds are avoided. However, the clearance of inulin is concentration dependent at low plasma concentrations, and at these levels, both the injection and constant infusion methods are inaccurate for measuring the glomerular filtration rate.

CHAPTER THREE

SINGLE INJECTION METHOD FOR MEASUREMENT OF RENAL BLOOD FLOW USING p-AMINOHIPPURIC ACID

INTRODUCTION

At low plasma concentrations p-aminohippuric acid (PAH) is almost completely extracted during a single passage through the kidney by active tubular transport, consequently, its renal clearance is widely used as a measure of renal plasma flow.

p-Aminohippuric acid was first used to measure renal plasma flow (RPF) in 1945 (Smith et al, 1945), and it has since become the standard for this purpose. The renal clearance of PAH is usually measured at steady state plasma concentrations, during constant intravenous infusion. As noted previously (p 47), this technique is tedious and cumbersome for both patient and investigator, and accurately timed complete urine collections are required. These problems limit this technique mainly to research applications. Radiolabelled substances such as I^{125} hippuran and I^{131} diodrast have been introduced as alternatives for clinical use, and their clearances have been calculated from plasma concentration-time decay curves, following single intravenous bolus administration. The total body clearance of hippuran is less than the simultaneously measured renal clearance of PAH, and it falls with time (Pihl, 1973; Pearson, 1979). Radioactive iodine is potentially hazardous, although uptake into the thyroid gland can be inhibited by prior administration of unlabelled iodine (i.e potassium iodate). A simple method for the measurement of renal blood flow is required, which does not have the problems associated with radioactivity.

Simplification of the measurement of the renal clearance of PAH to allow a clinically practicable method has been attempted using constant infusion techniques without urine collection, and single injection techniques with, and without urine collection. These have not been successful. The total body clearance of PAH is significantly greater than the corresponding

renal clearance (Statius Van Eps et al, 1967; Cole et al,1972). This has been attributed to metabolism of PAH as, the total body and renal clearances became equal after hydrolysis of the samples (Berger et al, 1948). PAH is metabolised to some extent to the N4 acetyl derivative (AcPAH) in animals and man (Smith, 1951), but this metabolic loss is considered to be insignificant, and to occur extrarenally. Thus it should not interfere with the estimation of RPF using the constant infusion method, or techniques in which urine PAH is determined (Smith et al, 1945; Pearson, 1979). However, if metabolism occurs in the kidney, the clearance and RPF will be underestimated. The renal metabolism of PAH has been reported in many mammals (except the dog), both "in vivo" and "in vitro" (Setchell & Blanch, 1961; Gyrd-Hansen & Rasmussen, 1970; Malyusz et al, 1979; Carpenter & Mudge, 1980). In man, "in vitro" metabolism of PAH has been shown to occur in kidney cortical slices (Frindt & Vial, 1968), and it may also occur "in vivo" in healthy individuals (Newman et al, 1949) and patients with various disorders (Grindt et al, 1974). The methods used for analysis of PAH in these studies were not specific, and the need for hydrolysis of AcPAH before estimation as total PAH in plasma and urine, make these results unreliable (Brown et al, 1976). There is no information on the kinetics of AcPAH after a single injection or constant intravenous infusion of PAH in normal volunteers, using a specific method of analysis.

SECTION I

ANALYTICAL METHODS

High Performance Liquid Chromatographic estimation of p-aminohippuric acid in plasma and urine.

p-Aminohippuric acid (PAH) is routinely determined by the Bratton & Marshall method (1939), with modifications (Smith et al, 1945; Harvey & Brothers, 1962). The method depends on the reaction of N-(-1-napthyl) ethylenediamine dihydrochloride with diazotizable aryl amines (i.e PAH, sulphonamides, p-aminobenzoic acid) to form azo dyes, which are measured colorimetrically. The reaction of primary aromatic amines with p-dimethylaminobenzaldehyde to form shiff bases, has also been utilised (Brun, 1951). These colorimetric methods are not specific (Waugh & Beall, 1974). They are also time consuming due to the need for multiple steps, which require careful timing (Shoup & Kissinger, 1975).

More specific techniques became possible with the development of high performance liquid chromatography (HPLC). Methods for the assay of PAH using HPLC with ultraviolet absorbance detection of PAH have been reported (Brown et al, 1976; Carpenter & Mudge, 1980; Meerdink et al, 1981; Prueksaritanont et al, 1984; Baranowski & Westenfelder, 1986), as well as with electrochemical detection (Shoup & Kissinger, 1975). A capillary gas chromatographic method has also been described (Libeer et al, 1981). The majority of these techniques are designed to measure not only PAH but also iothalamate, p-aminobenzoic acid and creatinine. Consequently, ultraviolet absorbance at 230 nm and 254 nm have been measured, but the maximum absorbance of PAH is at 280 nm (Moffat et al, 1986). The method described below is specific for PAH in plasma and urine. p-Aminobenzoic acid is used as an internal standard, and absorbance is measured at 280 nm.

Materials

Sodium aminohippurate (20% w/v) was obtained from

Merck Sharp & Dohme, Hoddesdon, p-aminohippuric acid from Sigma, Poole, Dorset and potassium nitrate (KNO3), p-aminobenzoic acid (PABA), perchloric acid (HCl04), gentisic acid and sulphanilamide were all purchased from British Drug House, Poole, Dorset. Acetonitrile (HPLC grade) was obtained from FSA Laboratory Supplies, Loughborough and glacial acetic acid, from Thornten & Ross Ltd, Huddersfield. The mobile phase was degassed using Helium gas, obtained from the British Oxygen Company.

Instrumentation

The HPLC system consisted of a solvent delivery pump (Altex 110), a WISP 710 B (Waters Intelligent Sample Processor) auto sample injector. A filter and a Waters "Guard-PAK" precolumn packed with the same material as the analytical column, was fitted between the injector and the column. The analytical column was an 8 mm x 10 cm Waters "Radial-PAK" Cartridge containing a reverse phase, 5 µ or 10 µ C18 bonded spherical silica support in a Waters RCM-100 Radial Compression Module. A Waters series 441 ultraviolet (UV) absorbance detector or a Perkin-Elmer Model LC-75 detector, were used at 280 nm. The UV detector output was passed to a chart recorder or a Shmadzu (C-R1B) integrator, to produce chromatograms and peak heights.

Plasma p-aminohippuric acid assay

Chromatographic conditions

Plasma p-aminohippuric acid was estimated (5 µ column) under the following chromatographic conditions:

Mobile Phase : 0.005 M potassium nitrate containing 2 % acetic acid : acetonitrile (195:11.5 V/V)
Flow Rate : 2 ml/min
Pressure : 1000 psi
Wavelength : 280 nm (detector)

Preparation of standards and samples

Serial dilutions of a stock solution of p-aminohippuric acid in fresh blank plasma were prepared to give concentrations ranging from 1 - 50 mg/l, to run with the unknown samples. To 250 µl of plasma in a plastic tube, 250 µl of aqueous internal standard containing 20 mg/l p-aminobenzoic acid was added. (500 mg/l of gentisic acid in aqueous solution can be used as an alternative internal standard). Fifty µl of 24 % perchloric acid was then added with continuous mixing (Whirlimix, Jencons Scientific), to precipitate the plasma proteins. The samples were centrifuged at 1400g for 10 minutes and up to 40 µl of clear supernatant was injected onto the column.

Calculation of results

The peak height ratios (PAH divided by p-aminobenzoic acid) were measured for the standards, and plotted against the known standard concentrations. The peak height ratios of the unknown samples were read off the standard plot, to give a concentration in mg/l.

Results

Chromatograms from drug-free plasma, and from plasma obtained 10 minutes after an intravenous bolus injection of sodium aminohippurate are shown in Fig 3.1.1. The retention times of p-aminohippuric acid, p-aminobenzoic acid and gentisic acid were 2.92, 4.39 and 7 mins respectively. The limit of detection for p-aminohippuric acid was less than 1 mg/l and no interference was observed with the drugs used in the reported studies.

Validation

The linearity and precision were determined by replicate analyses of the standard solutions of p-aminohippuric acid on 5 occasions, over a period of one week.

The results are shown in Table 3.1.1 and Fig 3.1.2.

Fig 3.1.1

Chromatograms obtained from the assay of:-
A) Drug free plasma (25 µl injection)
B) Plasma sample obtained 10 minutes after an intravenous bolus dose of 723 mg of sodium aminohippurate. The sample contained 21.82 mg/l p-aminohippuric acid (P) plus 20 mg/l p-aminobenzoic acid (internal standard (IS)), and 500 mg/l gentisic acid (G), (20 µl injection). Detection was at 280 nm (0.05 AUFS) and 0 indicates the time of injection.

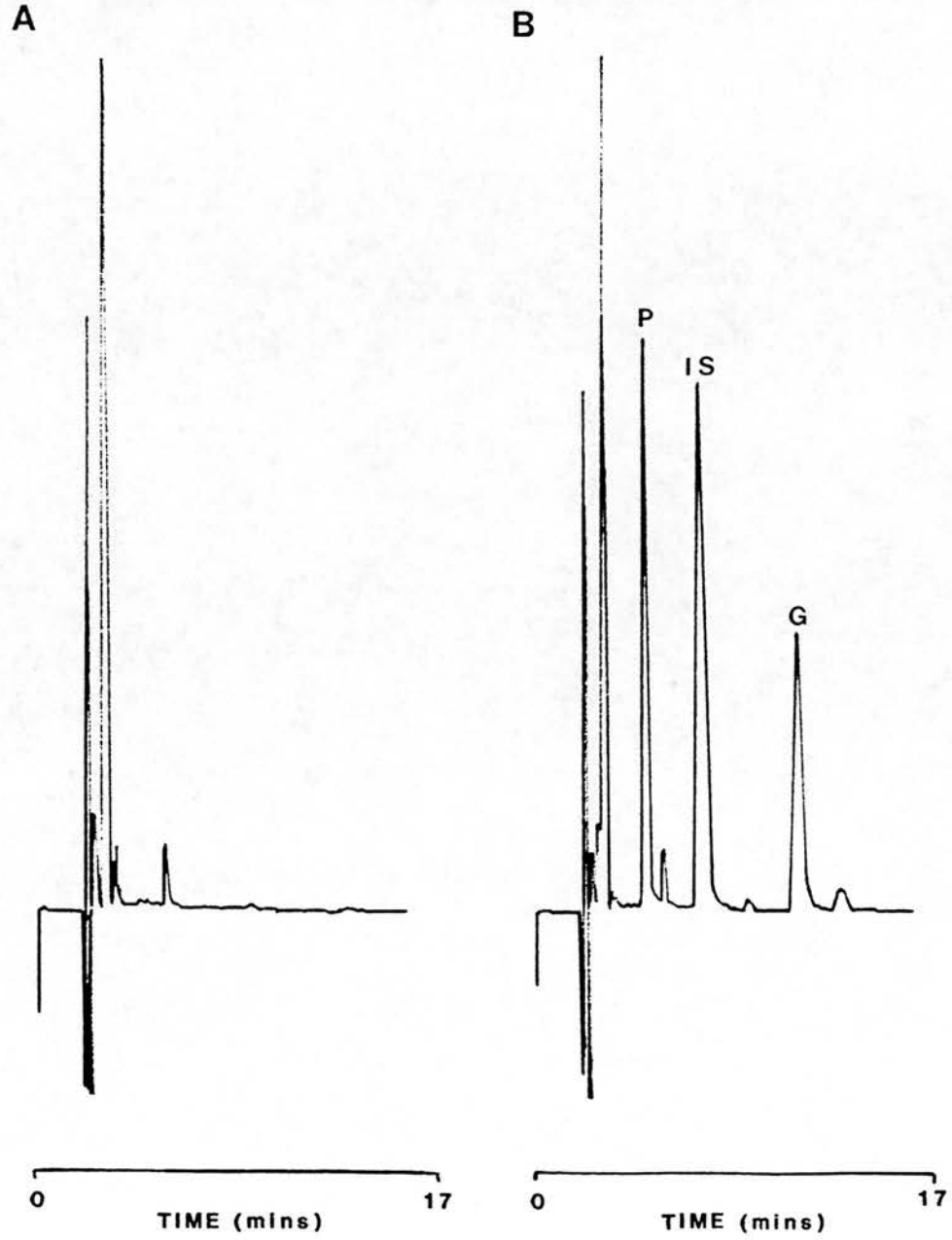


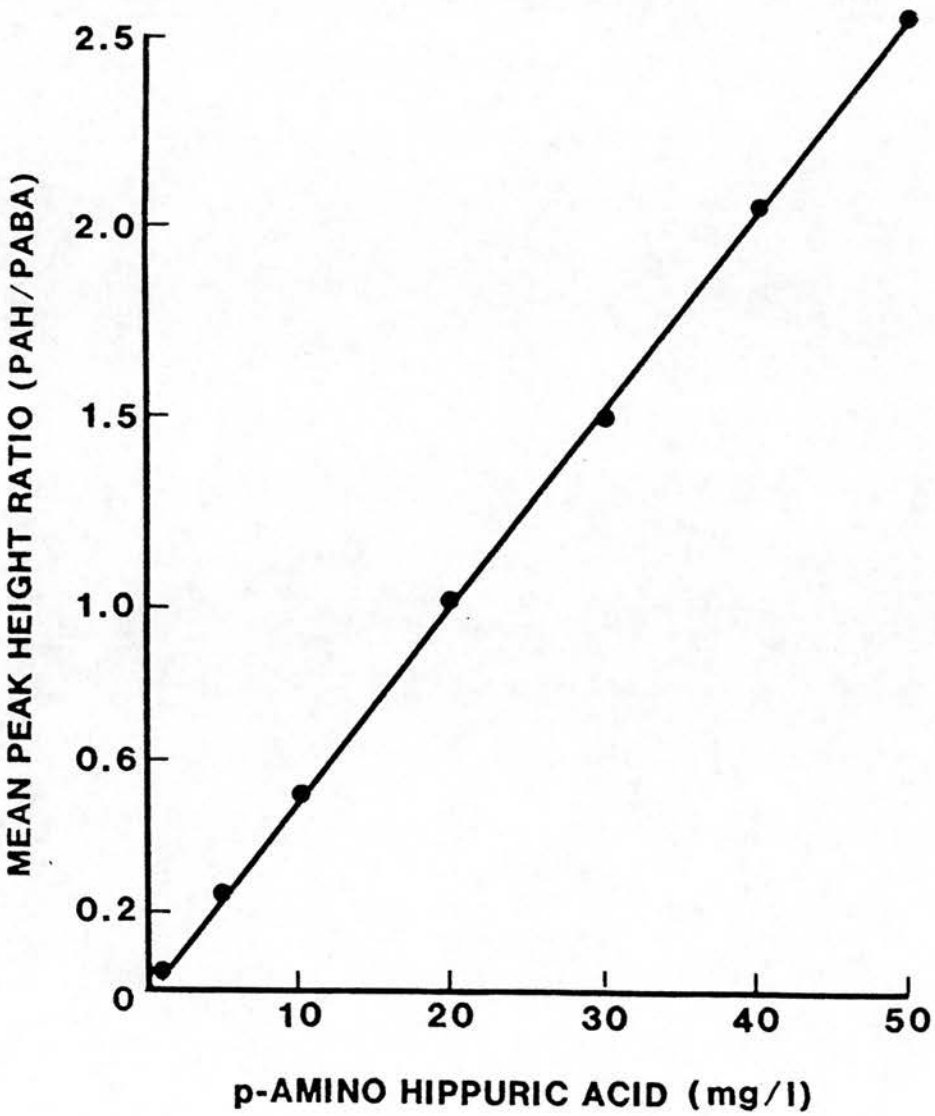
TABLE 3.1.1

Replicate HPLC analysis of p-aminohippuric acid in plasma (1 - 50 mg/l)

p-Aminohippuric acid Conc. mg/l	Peak Height Ratio (PAH/PABA) Run 1	Run 2	Run 3	Run 4	Run 5	Mean	Standard Deviation	Coefficient of Variation (%)
1	0.04	0.04	0.04	0.04	0.05	0.042	0.0045	10.6
5	0.24	0.24	0.24	0.22	0.24	0.236	0.0089	3.8
10	0.49	0.51	0.51	0.47	0.50	0.496	0.017	3.4
20	1.02	1.04	1.02	1.00	1.02	1.02	0.014	1.4
30	1.50	1.56	1.55	1.49	1.46	1.51	0.04	2.8
40	2.11	2.06	2.12	2.00	2.03	2.06	0.05	2.5
50	2.56	2.64	2.59	2.50	2.49	2.56	0.063	2.5

Fig 3.1.2

Calibration plot of the mean peak height ratios obtained following repeated analysis (N=5) of plasma containing p-aminohippuric acid in the concentration range 1-50 mg/l. PABA= p-aminobenzoic acid (the internal standard).



The coefficients of variation ranged from 10.8 % at 1 mg/l to less than 4 % for concentrations of 5 - 50 mg/l. The calibration plot calculated using linear regression was linear over the concentration range 1-50 mg/l, and passed close to the origin.

The mean recoveries of p-aminohippuric acid from spiked plasma compared with aqueous solution at concentrations of 30, 15 and 5 mg/l were 95, 93, and 97% respectively (Table 3.1.2).

p-Aminohippuric acid urine assay

Chromatographic conditions

The conditions tend to be varied to change the retention times of PAH and the internal standard, to avoid interfering peaks in the urine. p-Aminohippuric acid was determined in urine, using the same column as for plasma with the following chromatographic conditions:

Mobile Phase : 0.01 M potassium nitrate containing 1% acetic acid : acetonitrile (195:7 V/V)
Flow Rate : 2 ml/min
Pressure : 1000 psi
Wavelength : 280 nm (detector)

Preparation of standards and samples

Serial dilutions of a stock solution of p-aminohippuric acid in blank urine were prepared to give concentrations ranging from 50 - 800 mg/l, and these were run with the unknown samples. To 250 µl of appropriately diluted urine, 250 µl of aqueous internal standard was added containing 200 mg/l p-aminobenzoic acid. (200 mg/l sulphanilamide can be used as an alternative aqueous internal standard). The samples were mixed (Whirlimix, Jencons Scientific), and up to 15 µl injected directly on to the HPLC column.

TABLE 3.1.2

Mean relative recovery of p-aminohippuric acid from spiked plasma

p-aminohippuric acid Conc. mg/l	% of Aqueous sample peak height					Mean	Standard Deviation	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5			
30	95	95	94	95	96	95	0.81	0.85
15	92	95	94	90	-	93	1.80	2.00
5	91	97	98	94	102	96	4.10	4.20

Note: each percentage value is the mean from samples from three different subjects

Calculation of results

The peak height ratios (PAH divided by p-aminobenzoic acid) were measured for the standards and plotted against the known standard concentrations. The peak height ratios of the unknown samples were read of the standard plot to give concentrations in mg/l.

Results

Chromatograms from drug-free urine, and from urine obtained 2-2½ hours after the start of a constant intravenous infusion of sodium aminohippurate, are shown in Fig 3.1.3. The retention times of p-aminohippuric acid, p-aminobenzoic acid and sulphanilamide were 3.5, 5.5 and 2.8 minutes respectively. The limit of detection of p-aminohippuric acid depended on the presence of interfering peaks in the urine.

Validation

The results of 5 replicate analyses of standard solutions of p-aminohippuric acid in urine over a period of one week, are shown in Table 3.1.3 and Fig 3.1.4. The coefficients of variation were below 3 % for all concentrations. The calibration plot was linear over the concentration range studied, and passed close to the origin.

Discussion

p-Aminohippuric acid in plasma and urine was estimated directly by HPLC, using ultraviolet detection at 280 nm. The assays are rapid, simple and precise. p-Aminobenzoic is a potential metabolite of PAH. To rule out this possibility as a cause of error, samples also contained gentisic acid and sulphanilamide, as alternative internal standards for plasma and urine respectively. In no cases was there evidence for the presence of p-aminobenzoic acid other than as the internal standard.

Fig 3.1.3

Chromatograms obtained from the assay of:-
A) Drug free urine (15 µl injection)
B) Urine collected 2-2½ hours after the start of a constant infusion of sodium aminohippurate (15 mg/min). The sample contained 1280 mg/l p-aminohippuric acid (P), plus 200 mg/l p-aminobenzoic acid (IS) and 200 mg/l sulphanilamide (S), (8 µl injection)
Detection was at 280 nm (0.32 AUFS) and 0 indicates the time of injection.

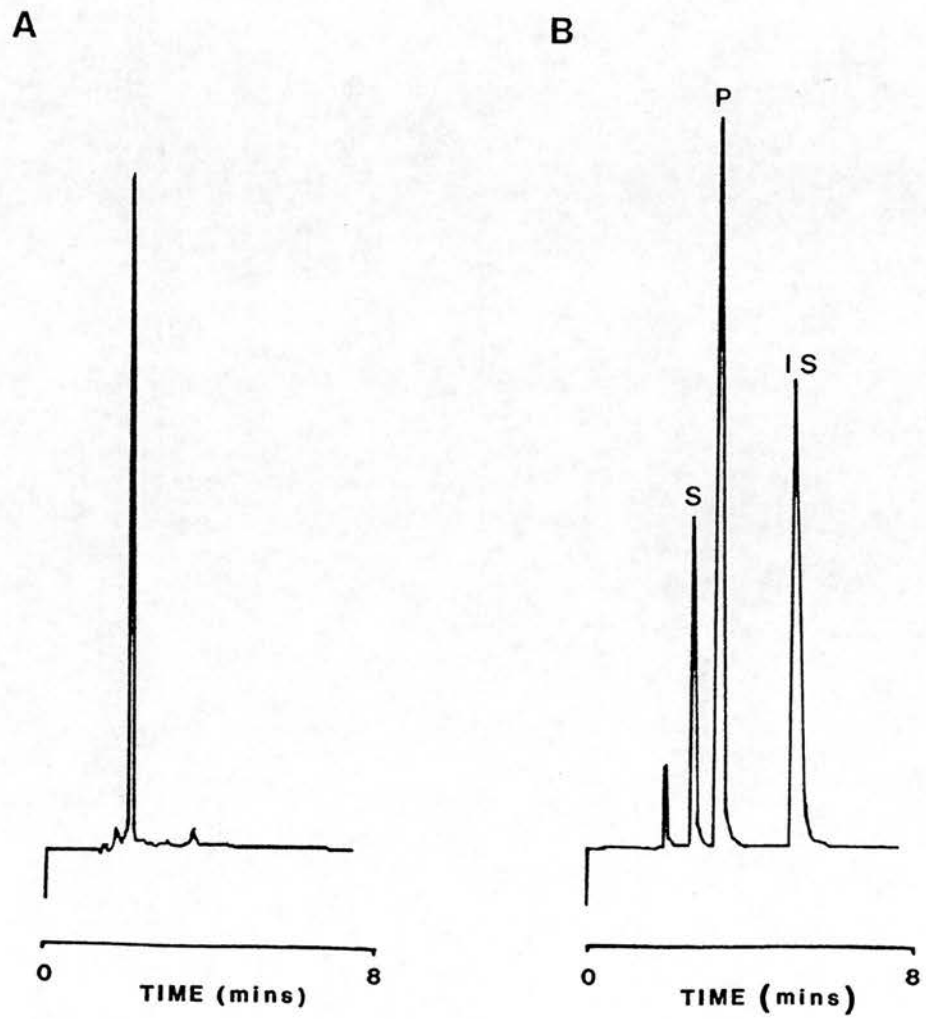


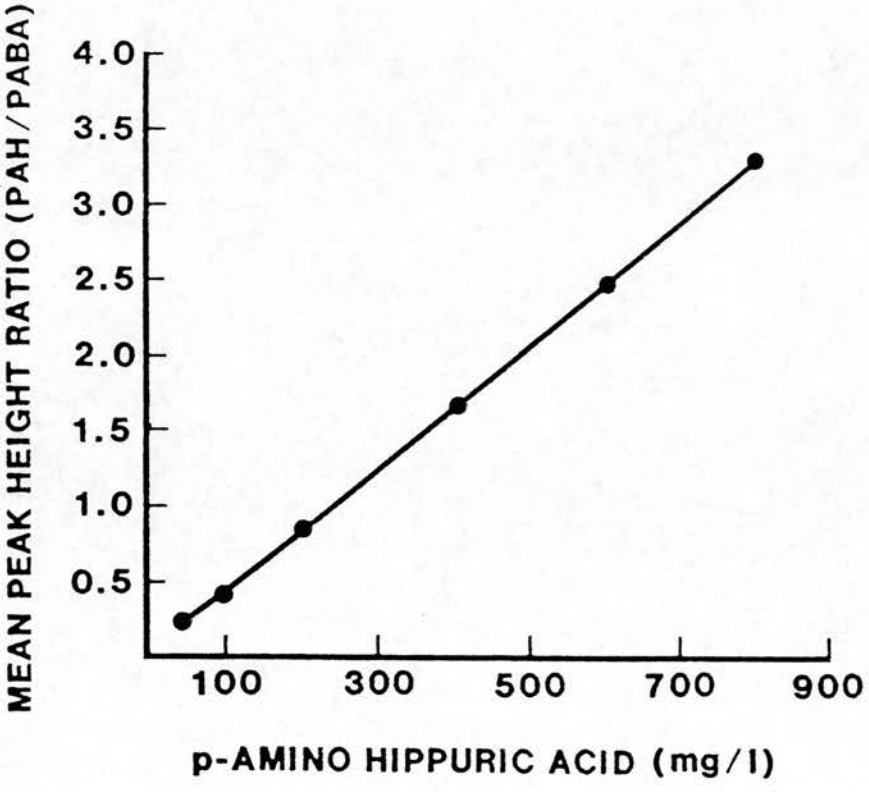
TABLE 3.1.3

Replicate analysis of p-aminohippuric acid in urine (1 - 50 mg/l) by HPLC

p-Aminohippuric acid Conc. mg/l	Peak Height Ratio (PAH/PABA) Run 1	Run 2	Run 3	Run 4	Run 5	Mean	Standard Deviation	Coefficient of Variation (%)
50	0.21	0.21	0.22	0.22	0.21	0.21	0.0055	2.6
100	0.43	0.43	0.44	0.44	0.42	0.43	0.0084	1.9
200	0.87	0.87	0.88	0.89	0.85	0.87	0.015	1.7
400	1.68	1.64	1.71	1.66	1.65	1.67	0.028	1.7
600	2.46	2.46	2.54	2.49	2.38	2.47	0.058	2.4
800	3.29	3.22	3.35	3.36	3.26	3.30	0.059	1.8

Fig 3.1.4

Calibration plot of the mean peak height ratios obtained following repeated analysis (N=5) of urine containing p-aminohippuric acid in the concentration range 50-800 mg/l. PABA= p-aminobenzoic acid (the internal standard).



High Performance Liquid Chromatographic estimation of acetyl p-aminohippuric acid in plasma and urine.

Acetyl p-aminohippuric acid (AcPAH) is the major metabolite of PAH and, it has usually been determined as PAH (see p 148) after removal of the acetyl group by hydrolysis, with concentrated hydrochloric acid at 96°C for 1 to 3½ hours (Smith et al, 1945; Newman et al, 1949; Statius Van Eps, 1967; Grindt et al, 1974). The AcPAH is represented as the difference in PAH concentrations before, and after hydrolysis. Treatment of AcPAH with hydrochloric acid also produces secondary products such as p-aminobenzoic acid, which also give a positive colour reaction and hence, a false estimate of AcPAH concentrations (Brown et al, 1976). Specific HPLC assays for AcPAH have therefore been developed, to overcome these problems (Brown et al, 1974; Meerdink et al, 1981; Stolk et al, 1985). The method of Brown et al, (1974), is not sensitive enough to detect AcPAH in plasma following intravenous administration of PAH. The other methods can measure AcPAH and PAH simultaneously, but this is impractical because of the large concentration differences in plasma. The HPLC method described here, can measure AcPAH without hydrolysis at low concentrations in plasma and urine.

Materials

Sodium aminohippurate, potassium nitrate, p-aminobenzoic acid (PABA), perchloric acid, acetonitrile, glacial acetic acid, and helium were obtained, as described previously (p 149). Anthranilic acid and hippuric acid were purchased from British Drug House, Poole, Dorset.

Acetyl-p-aminohippuric acid was synthesised by reacting PAH with acetic anhydride (May & Baker, Dagenham, U.K.) using the method of Newman et al, 1949. A 20 % solution of sodium aminohippurate was diluted

with an equal volume of distilled water. 2 mols acetic anhydride, was added whilst stirring to 1 mol PAH. The solution was allowed to stand at room temperature for 30 minutes, with occasional shaking, before the mixture was cooled in an ice bath, and filtered through a Bruchner funnel by suction. The precipitate was washed several times with ice cooled distilled water, and then with 95 % ethyl alcohol. The precipitate was left to dry protected from light. The AcPAH was a creamy crystalline substance, with a melting point of 243.3°C to 247.6°C. This was similar to the melting point of 242°C reported by Stolk et al, (1985) but higher than 209 to 228°C described by Statius Van Eps et al, (1967). The identity of the AcPAH was confirmed by infra red spectrophotometry, nuclear magnetic resonance and mass spectrophotometry (MW = 235), at the Chemistry Department of Edinburgh University. Only one peak was observed on HPLC.

Instrumentation

The HPLC system was as described on page 149.

Acetyl-p-aminohippuric acid plasma assay

Chromatographic conditions

Plasma acetyl-p-aminohippuric acid was estimated using a Radial-PAK, C18, 5 μ column under the following chromatographic conditions:

Mobile Phase : 0.01 M potassium nitrate containing 1% acetic acid : acetonitrile (95:7 V/V)
Flow Rate : 2 ml/min
Pressure : 1000 psi
Wavelength : 280 nm (detector)

Preparation of standards and samples

Serial dilutions of a stock solution of acetyl-p-aminohippuric acid in blank plasma were prepared in the concentration range of 0.5 - 4 mg/l. These standard

solutions were run with the unknown samples. To 250 µl of plasma in a plastic tube, 100 µl of aqueous internal standard containing 250 mg/l hippuric acid was added. (2.5 mg/l of aqueous p-aminobenzoic acid may be used as an alternative). 50 µl of 24% perchloric acid was added with continuous mixing (Whirlimix, Jencons Scientific) to precipitate the plasma proteins. The samples were centrifuged at 1400g for 10 minutes and up to 90 µl of clear supernatant was injected onto the HPLC column.

Calculation of results

The peak height ratios (AcPAH divided by hippuric acid) were measured for the standards and plotted against the known standard concentrations. The peak height ratios of the unknown samples were read off the standard plot to give concentrations in mg/l.

Results

Chromatograms from drug-free plasma and from plasma obtained 2 hours after the start of a constant infusion of sodium aminohippurate are shown in Fig 3.1.5. The retention times of AcPAH, hippuric acid and p-aminobenzoic acid were 8, 11.5 and 5 minutes respectively. The limit of detection for AcPAH was less than 0.5 mg/l and no interference was obtained from tenoxicam used in the studies.

Validation

The linearity and reproducibility for the plasma assay were determined by replicate analysis of standard solutions of AcPAH on 5 occasions, over a period of one week (Table 3.1.4 and Fig 3.1.6). The coefficients of variation were below 3 % for all concentrations. The calibration plot was linear over the concentration range studied, and passed close to the origin. The mean recoveries of AcPAH, from spiked plasma, compared with aqueous samples containing 0.5, 1 and 5 mg/l were 89,

Fig 3.1.5

Chromatograms obtained from the assay of:-
A) Drug free plasma (55 µl injection)
B) Plasma sample obtained 2 hours after the start of a constant infusion of sodium aminohippurate (15 mg/min). The sample contained 2.8 mg/l acetyl-p-aminohippuric acid (A) plus 250 mg/l hippuric acid (IS), 2.5 mg/l p-aminobenzoic acid (PB), and p-aminohippuric acid (P) (40 µl injection).
Detection was at 280 nm (0.02 AUFS), and 0 indicates the time of injection.

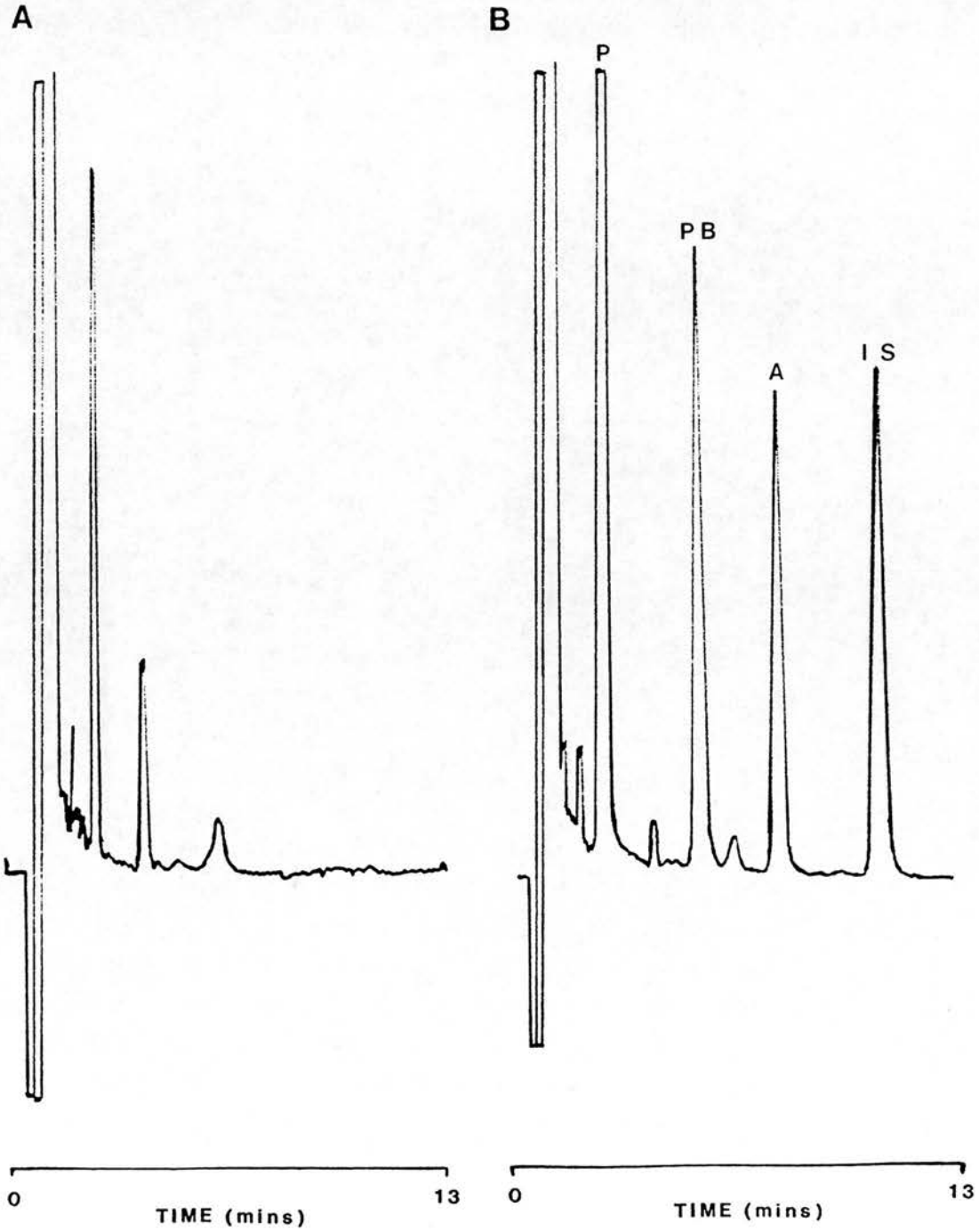


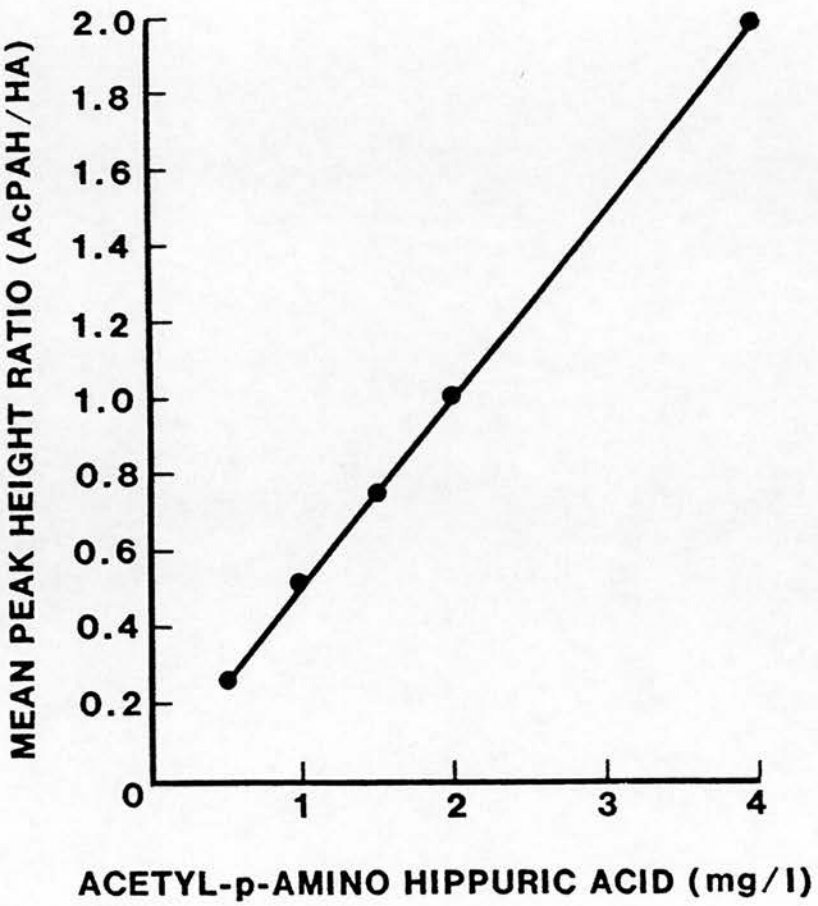
TABLE 3.1.4

Replicate HPLC analysis of Acetyl- p-aminohippuric acid in plasma (0.5 - 4 mg/l)

Acetyl-p-aminohippuric acid Conc. mg/l	Peak Height Ratio (AcPAH/HA) Run 1	Run 2	Run 3	Run 4	Run 5	Mean	Standard Deviation	Coefficient of Variation (%)
0.5	0.26	0.26	0.26	0.26	0.25	0.26	0.0045	1.7
1.0	0.51	0.53	0.53	0.50	0.51	0.52	0.013	2.6
1.5	0.75	0.76	0.78	0.73	0.74	0.75	0.019	2.6
2.0	1.01	1.03	1.03	0.98	1.00	1.01	0.021	2.1
4.0	2.06	1.93	2.00	1.98	1.98	1.99	0.047	2.4

Fig 3.1.6

Calibration plot of the mean peak height ratios obtained following repeated analysis (N=5) of plasma containing acetyl-p-aminohippuric acid in the concentration range 0.5-4 mg/l. HA= hippuric acid (the internal standard).



82, and 89 % respectively (Table 3.1.5).

Acetyl-p-aminohippuric acid urine assay

Chromatographic conditions

Acetyl-p-aminohippuric acid was determined in urine using a Radial-PAK, C18, 10 µ column, under the following chromatographic conditions:

Mobile Phase : 0.01 M potassium nitrate containing 1% acetic acid : acetonitrile (92:8 V/V)
Flow Rate : 1.9 ml/min
Pressure : 1000 psi
Wavelength : 280 nm (detector)

Preparation of standards and samples

Serial dilutions of a stock solution of acetyl-p-aminohippuric acid in blank urine were prepared to give concentrations ranging from 50 - 250 mg/l. These were run with the unknown samples. To 250 µl of appropriately diluted urine, 175 µl of aqueous internal standard containing 50 mg/l p-aminobenzoic acid was added. (1250 mg/l aqueous anthranilic acid may be used as an alternative). The samples were mixed (Whirlimix, Jencons Scientific), and up to 20 µl injected directly on to the HPLC column.

Calculation of results

The peak height ratios (AcPAH divided by p-aminobenzoic acid) were measured for the standards, and plotted against the known standard concentrations. The peak height ratios of the unknown samples were read off the standard plot, to give concentrations in mg/l

Results

Chromatograms from drug-free urine, and from urine obtained 2-2½ hours after the start of a constant infusion of sodium aminohippurate, are shown in Fig 3.1.7. The retention times of AcPAH, p-aminobenzoic acid

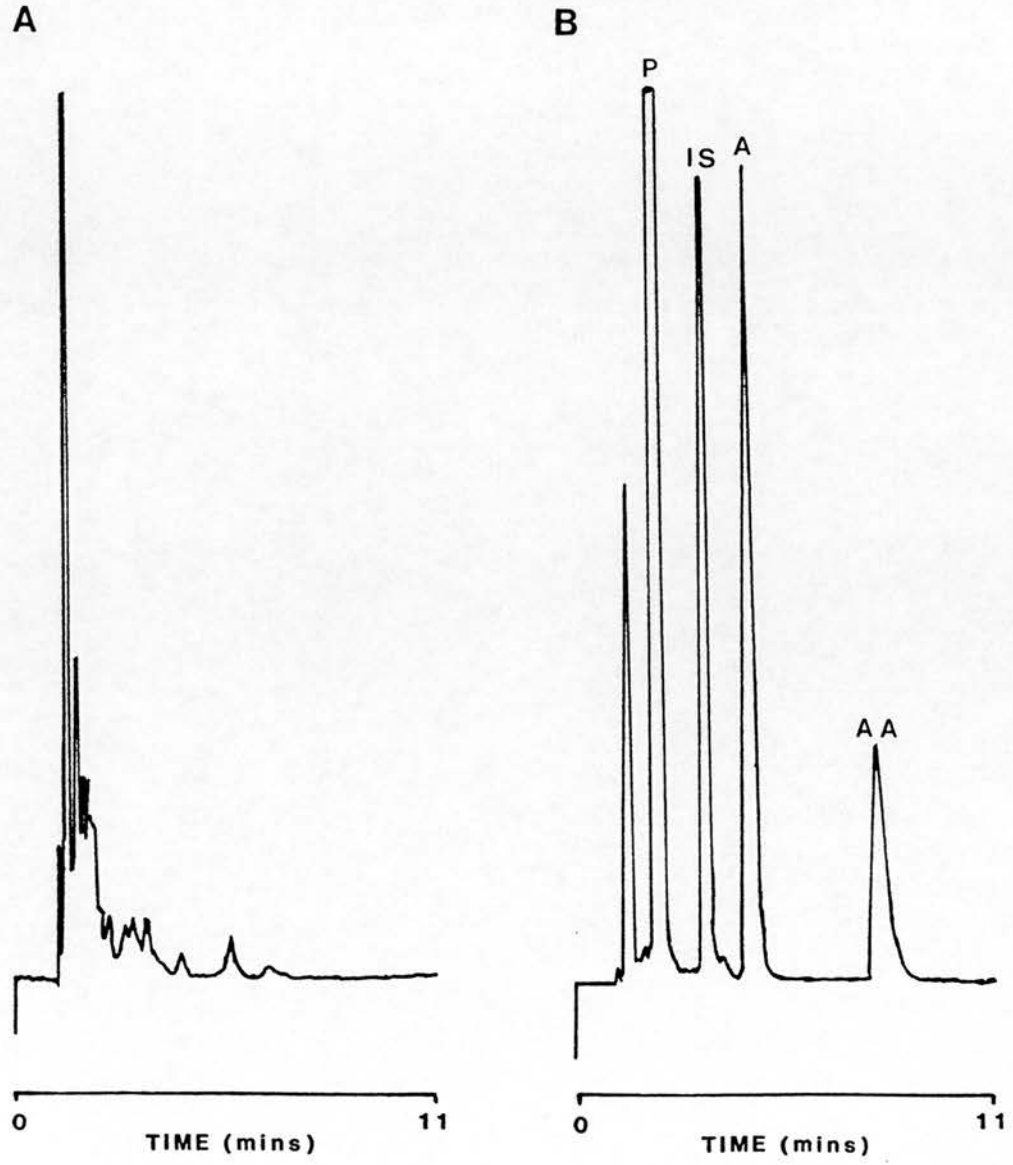
TABLE 3.1.5

Mean relative recovery of acetyl-p-aminohippuric acid from spiked plasma

Acetyl-p-aminohippuric acid Conc. mg/l	% of Aqueous sample peak height					Mean	Standard Deviation	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5			
5.0	90	90	89	89	90	90	0.55	0.61
1.0	83	81	83	81	82	82	1.00	1.22
0.5	89	91	87	88	89	89	1.48	1.67

Fig 3.1.7

Chromatograms obtained from the assay of:-
A) Drug free urine (20 µl injection)
B) Urine collected 2-2½ hours after the start of a constant infusion of sodium aminohippurate (19 mg/min). The sample contained 229 mg/l acetyl-p-aminohippuric acid (A) plus 50 mg/l p-aminobenzoic acid (IS), 1250 mg/l anthranilic acid (AA), and p-aminohippuric acid (P) (20 µl injection).
Detection was at 280 nm (0.1 AUFS), and 0 indicates the time of injection.



and anthranilic acid were 4.5, 3.4 and 8.1 minutes respectively. The limit of detection for AcPAH depends on the presence of interfering endogenous peaks.

Validation

The standard solutions of AcPAH in urine were run through the procedure 5 times, and the results are shown in Table 3.1.6 and Fig 3.1.8. The coefficients of variation were below 3 % for all the standards. The calibration plot was linear over the concentration range studied and passed close to the origin.

Discussion

The method allows rapid, direct, simple and precise assay of acetyl-p-aminohippuric acid in plasma and urine, without the need for prior hydrolysis. AcPAH was found to disappear slowly, in the presence of perchloric acid, after 10 hours (data not shown). Samples must therefore be processed within this time, after the addition of perchloric acid. p-Aminobenzoic and hippuric acid are potential metabolites of PAH. To rule out this possibility as a cause of error, urine samples also contained anthranilic acid, as an alternative internal standard. However, in no cases was there evidence for the presence of p-aminobenzoic acid or hippuric, acid other than as the internal standard.

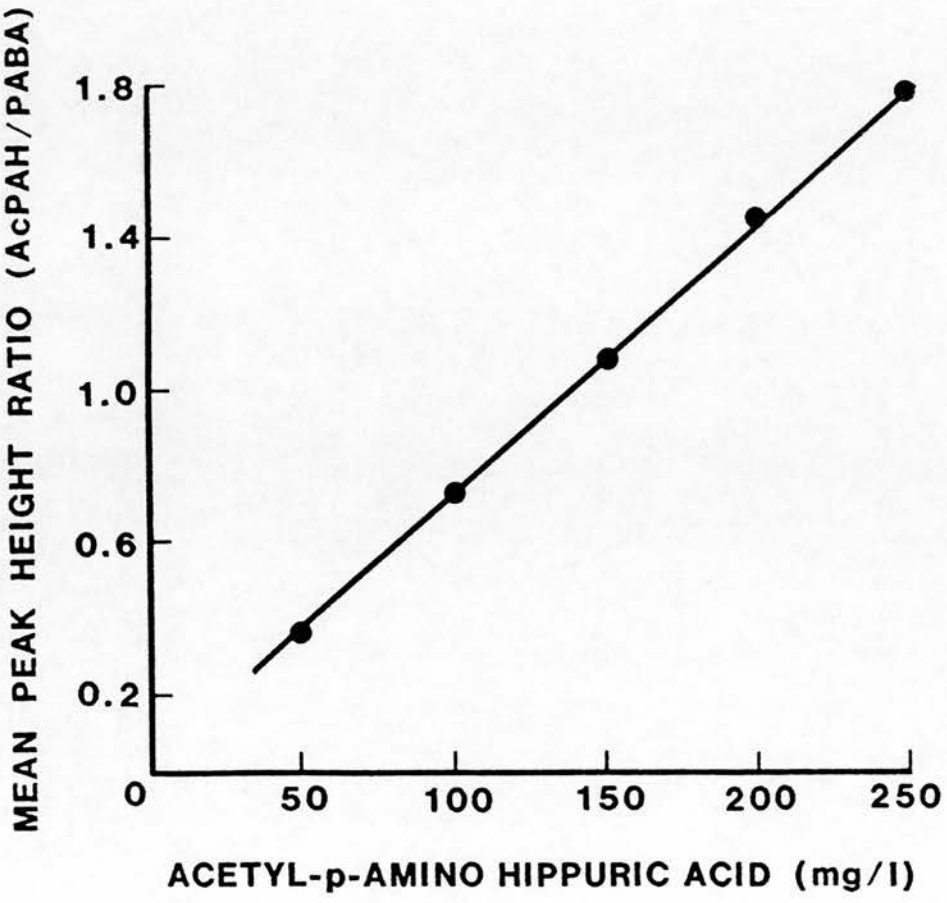
TABLE 3.1.6

Replicate analysis of Urine containing Acetyl-p-aminohippuric acid (0.5 - 4 mg/l) by HPLC

Acetyl-p-aminohippuric acid Conc. mg/l	Peak Height Ratio (AcPAH/PABA) Run 1	Run 2	Run 3	Run 4	Run 5	Mean	Standard Deviation	Coefficient of Variation (%)
50	0.38	0.36	0.36	0.36	0.36	0.36	0.0089	2.5
100	0.74	0.73	0.71	0.76	0.71	0.73	0.02	2.9
150	1.13	1.07	1.05	1.07	1.09	1.08	0.03	2.6
200	1.48	1.43	1.45	1.47	1.41	1.45	0.03	2.0
250	1.84	1.78	1.74	1.82	1.73	1.78	0.05	2.7

Fig 3.1.8

Calibration plot of the mean peak height ratios obtained following repeated analysis (N=5) of urine containing acetyl-p-aminohippuric acid in the concentration range 50-250 mg/l. PABA= p-aminobenzoic acid (the internal standard).



SECTION II

THE DISPOSITION AND KINETICS OF PAH FOLLOWING A SINGLE INTRAVENOUS INJECTION AND AFTER CONSTANT INFUSION

INTRODUCTION

The object of this study was to investigate the disposition and kinetics of PAH and AcPAH after a single injection, and during constant infusion of PAH in healthy male volunteers, using a direct and specific assay of PAH and AcPAH. It was also hoped to be able to develop a single injection technique for the estimation of RPF, without dependence on urine collections. The clearance of PAH following single injection and during constant infusion was compared in the same individuals.

METHODS

Single injection

The total body and renal clearances of PAH and AcPAH renal clearances were measured in 26 healthy male individuals, with a mean age 27 $\pm$ 6 years (range 21-50 yrs) weighing 71 $\pm$ 7 Kg (range 57-89 Kg), following a single intravenous bolus injection of PAH.

The initial procedure was described in chapter two (p 50). Sodium aminohippurate (10 mg/Kg, 5 ml, 20 % w/v solution, Merck Sharp & Dohme, Hoddesdon, UK) was given as an intravenous bolus injection over 1 minute, prior to inulin administration (p 50). Venous blood samples (10 ml) were collected at 3, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 180, 240, 360 and 480 minutes, after the end of the injection. Urine was collected hourly for the first four hours, then at 6, 8 and 24 hours. The syringe used to give PAH was weighed full before, and again empty after administration, to allow calculation of the exact dose.

Constant infusion

The PAH and AcPAH renal clearances were measured in ten healthy male volunteers, aged between 21-50 years (mean 27 $\pm$ 8 yrs) weighing 57-78 Kg (mean 71 $\pm$ 7 Kg) who received PAH by constant infusion. The single injection

and constant infusion methods were used, in random order, and the two studies were carried out within fourteen days of each other, except in the case of volunteer RJ, in whom the interval was 8 months.

The initial procedure was as described on page 49 chapter 2. A loading dose of Sodium PAH (440 mg, 2.2 ml, 20 % solution) was administered over one minute, followed by a maintenance infusion containing PAH (19 mg/ml) in 5 % dextrose, which was given simultaneously with inulin (p 49), at a rate of 1 ml/min. After an equilibrium period of one hour, the bladder was emptied and 4 half hourly timed urine samples were collected. Venous blood was drawn at the start and end of each urine collection period using the cannula which had not been used for the infusion.

Sample storage and analysis

The blood samples were collected in 10 ml lithium heparin tubes, and centrifuged at 1500 g for 10 minutes. The plasma was removed, and stored in 5 ml plastic tubes at -20°C, until analysed. Urine volume and pH (Radiometer) were measured, and a 20 ml aliquiot stored at -20°C until analysis.

Plasma and urine were analysed for PAH and AcPAH using HPLC as described in section I (p 147).

Data analysis

Single injection

The exact dose of sodium PAH was calculated by syringe weighing, as for inulin (chapter 2, p 53). The dose of sodium aminohippurate was then converted to the PAH equivalent by applying a correction factor of 1.113, based on the molecular weights of sodium aminohippurate (216), and PAH (194). The dose administered will subsequently be referred to as the amount of PAH.

The total body clearance of PAH was calculated from the plasma concentration-time data alone, in the same

manner as described for inulin (chapter 2, p 52), using data at 3, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120 minutes following the end of the PAH injection. In some cases, the three minute data was ignored as concentration were lower then at 5 minutes. After two hours, both plasma PAH and AcPAH concentrations were below the limit of measurement. The total body clearance was calculated using equation 8 (p 52) i.e. the administered dose divided by the area under the plasma concentration time curve (AUC), extrapolated to infinity. The total body clearance was calculated from 0-1 and 0-2 hours.

The renal clearances of PAH and AcPAH were estimated from equation 4 (p 53) i.e. the amount excreted divided by the corresponding AUC. The renal clearances of PAH and AcPAH were calculated for the 0-1 and 1-2 hour periods.

The percentage recovery of the dose of PAH was calculated for each urine collection period up to four hours, and summed to give the total recovery of PAH. The percentage recovery as AcPAH was calculated, after converting AcPAH to the PAH equivalent, by dividing by 1.216. This correction factor was obtained by dividing the molecular weight of AcPAH (236) by that of PAH (194). The recovery was calculated for each urine collection period up to eight hours, and summed to give the total percentage of the dose recovered as AcPAH.

The distribution half life for PAH was estimated in a similar manner to that described previously for inulin (chapter 2, p 90) for the 0-1 hour data. The plasma half life values of PAH and AcPAH ($t_{\frac{1}{2}}$) were calculated from the linear elimination phase of the plasma concentration time curve, using the following relationship:-

$$t_{\frac{1}{2}} = \frac{\log_{10} 2}{\text{slope}}$$

the half lives were estimated for each subject from 30

to 60 minutes for PAH, and 30 to 120 minutes for AcPAH.

Constant infusion

The renal clearances of PAH and AcPAH were calculated as described previously for inulin (chapter 2, p 52), for all urine collection periods.

All clearance values for PAH and AcPAH were corrected for a body surface area of 1.73 m^2.

Statistical methods

The significance of differences between means were determined using 2 way analysis of variance. The null hypothesis was rejected if $p<0.05$. Correlation coefficients were determined by linear regression.

RESULTS

Plasma concentrations

Individual PAH and AcPAH plasma concentrations are listed in Appendix I.

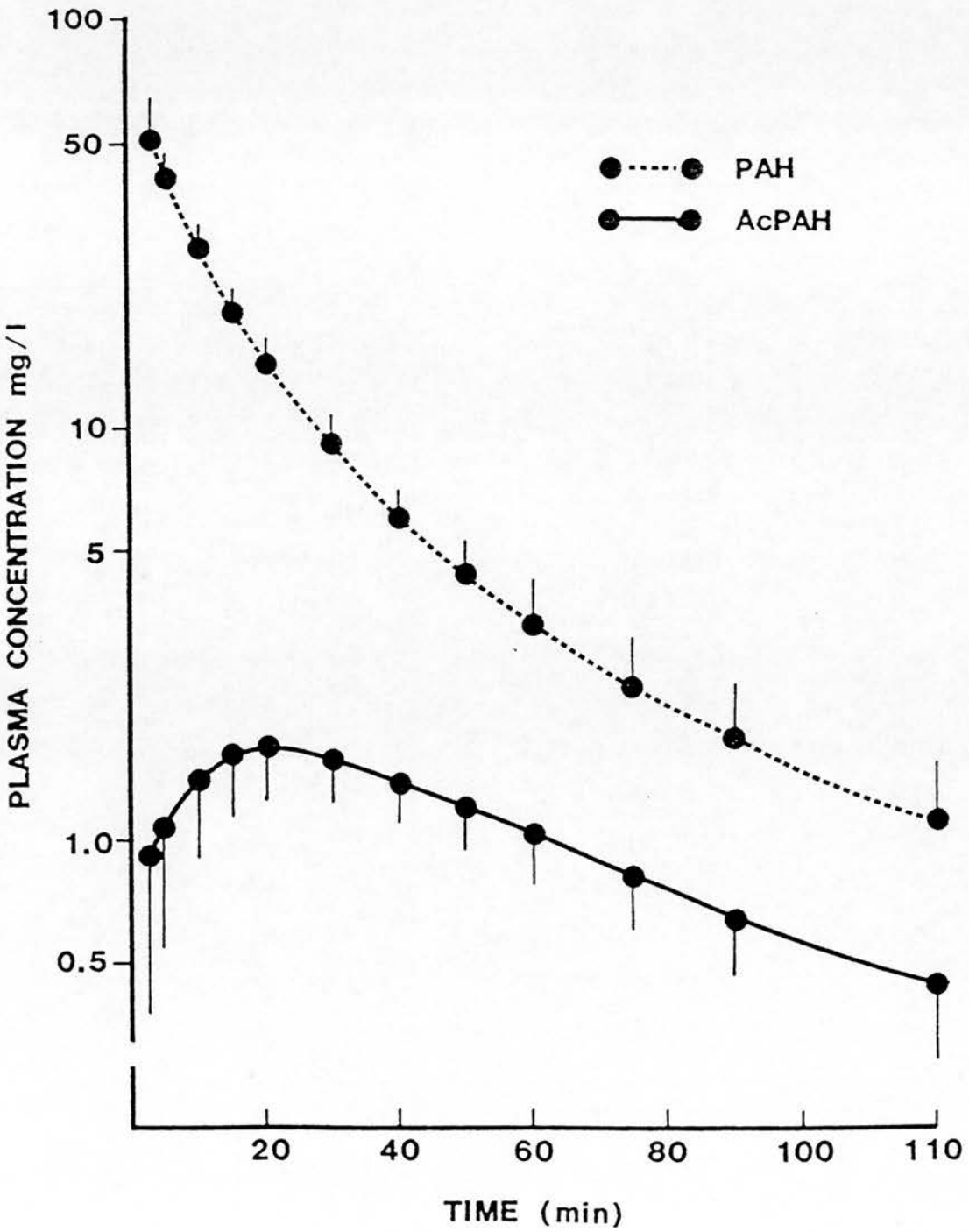
Single Injection

p-AMINOHIPPURIC ACID

Following intravenous bolus administration of PAH, the plasma concentration fell rapidly in all subjects in a curvilinear manner when plotted semilogarithmically against time (Fig 3.2.1). The plasma concentration time curve did not show a terminal linear phase but, the data up to one hour could be fitted to a two compartment model. The mean distribution half life calculated was 4.6 minutes, and therefore, distribution was on average more than 95 % complete 28 minutes after the intravenous injection. The mean elimination half life for PAH estimated from the 30 to 60 minute data was 21.9 $\pm$ 2.8 minutes (range 17-28 min) (Table 3.2.1). The mean PAH plasma concentration fell from 50.4 $\pm$ 13 mg/l at 3 minutes to 1.1 $\pm$ 0.4 mg/l at 120 minutes.

Fig 3.2.1

Mean plasma concentrations of PAH and AcPAH following an intravenous bolus injection of PAH (10 mg/Kg) in 26 healthy males. Bars = SD.



ACETYL-p-AMINOHIPPURIC ACID

Following an intravenous bolus administration of PAH, AcPAH was already present in plasma at 3 minutes, and a mean peak plasma concentration of 1.66 ± 0.4 mg/l was achieved by 20 minutes. Plasma concentrations declined progressively in a linear fashion after 40 minutes when plotted semilogarithmically against time (Fig 3.2.1). The mean elimination half life ($t_{\frac{1}{2}}$) estimated from the terminal linear elimination phase was 48 ± 10 minutes (range 33-76 min) (Table 3.2.2).

Constant infusion

p-AMINOHIPPURIC ACID

Steady state plasma concentrations were achieved in all subjects within 1½-2 hours of starting the infusion. The mean plasma concentrations after equilibration ranged from 26.4 ± 3 mg/l at 1 hour to 30.5 ± 2 mg/l at 3 hours (Fig 3.2.2).

ACETYL-p-AMINOHIPPURIC ACID

Plasma concentrations of AcPAH increased during the constant infusion of PAH, and became constant at 2 hours after the start (Fig 3.2.2). The mean plasma concentrations ranged from 2.7 ± 0.4 mg/l at 1 hour, to 3.3 ± 0.5 mg/l at 3 hours. These are some 10 times lower than the corresponding PAH plasma concentrations.

Clearances

Single injection

p-AMINOHIPPURIC ACID - RENAL CLEARANCE

The individual renal clearances of PAH for each collection period are given in Table 3.2.1. The mean renal clearance of PAH fell by 54 % during the first two hours. This fall was significant with respect to time, and plasma concentration ($p<0.01$). The mean renal clearances of PAH were 526 and 241 ml/min/1.73 m^2 over

Fig 3.2.2

Mean plasma concentrations of PAH and AcPAH during constant infusion of PAH in 10 healthy male subjects. Bars = ± SD.

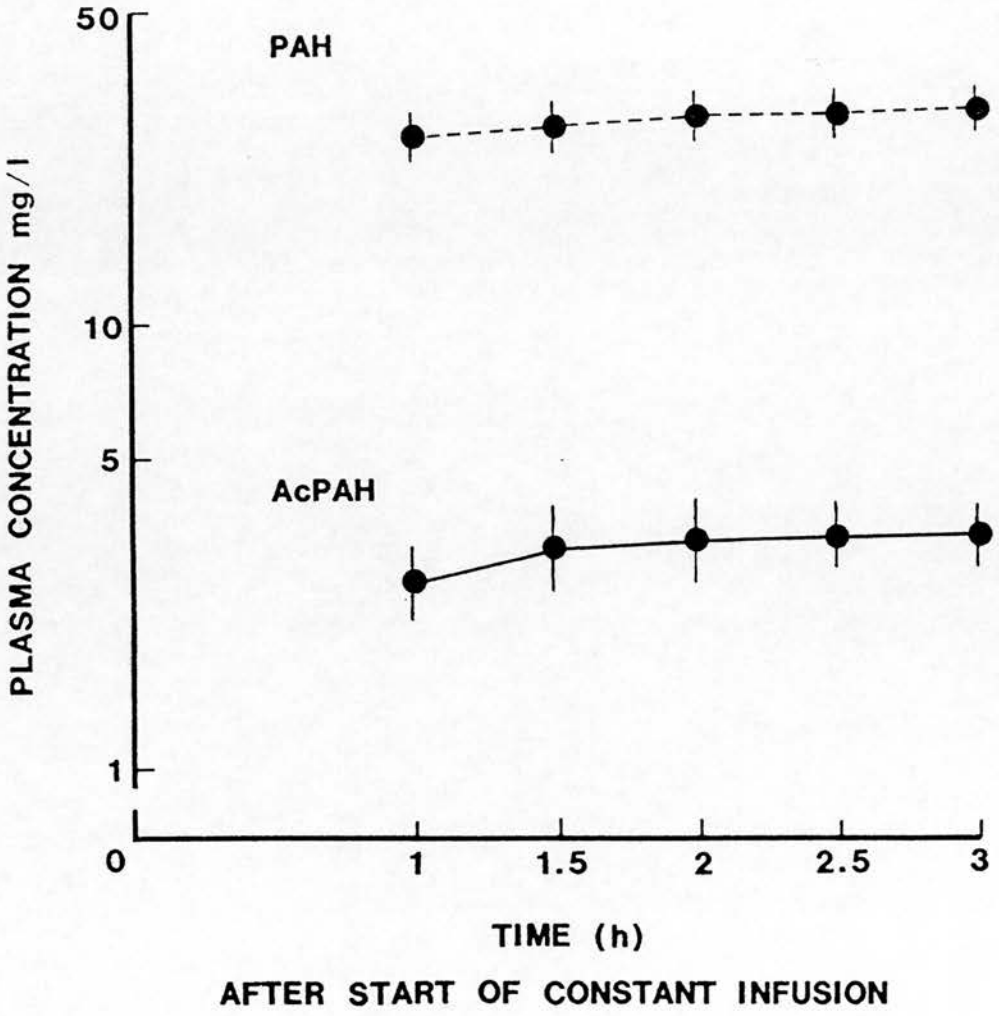


TABLE 3.2.1

The total body and renal clearances of PAH (ml/min/1.73 m^2) following a single intravenous bolus injection of PAH in 26 healthy males. The elimination half life $t_{\frac{1}{2}}$ is also given.

	RENAL CLEARANCE (h)		TOTAL BODY CL (h)		
SUBJECT	0-1	1-2	0-1	0-2	$t_{\frac{1}{2}}$ (min)
GM	479	233	581	528	20.7
AT	619	347	717	714	18.5
EC	526	233	612	571	21.9
WW	495	148	554	516	21.8
AB	540	257	535	552	19.8
RJ	600	196	631	631	18.2
JG	649	257	700	673	20.2
AD	803	258	836	817	16.8
AH	545	177	580	562	22.8
SB	476	165	575	520	21.2
JA	605	277	641	591	20.6
PD	451	165	531	476	27.9
SA	623	204	625	602	20.7
RF	513	198	586	554	27.5
MS	533	135	634	537	23.0
PF	581	278	669	605	17.2
TM	589	201	646	608	21.3
MK	451	599	602	575	19.7
DM	532	277	642	632	26.4
JN	478	210	585	540	23.4
BH	414	337	482	508	23.5
BS	415	262	456	482	23.8
GS	460	305	573	539	20.8
LP	497	255	640	601	22.9
RM	420	122	526	497	23.3
CP	384	178	451	416	24.5
MEAN	526	241	600	571	21.9
$\pm$SD	92	94	83	81	2.8

period 0-1 and 1-2 hours respectively (Fig 3.2.3).

p-AMINOHIPPURIC ACID - TOTAL BODY CLEARANCE

The individual total body clearances of PAH are given in Table 3.2.1. The mean total body clearance of PAH also fell from 0-1 to 0-2 hours, but only by 5 %. This fall was also significant with respect to time and plasma concentration ($p<0.01$). The mean total body clearances were 600 and 571 ml/min/1.73 m^2 for 0-1 and 0-2 hours, respectively (Fig 3.2.3).

Comparison of total body and renal clearance of PAH.

The 0-1 hour total body clearance of PAH exceeded the 0-1 hour renal clearance of PAH by a mean of 14 % ($p<0.01$; 600 vs 526 ml/min/ 1.73 m^2). There was a significant correlation between the 0-1 hour, total body and renal clearances of PAH ($r=0.903$, $p<0.001$, Fig 3.2.4) but not between 0-2 hour total body clearance and 1-2 hour renal clearance.

ACETYL-p-AMINOHIPPURIC ACID - RENAL CLEARANCE

The individual renal clearances of AcPAH following an intravenous bolus injection of PAH are given in Table 3.2.2 and the mean clearances were 806 and 891 ml/min/ 1.73 m^2 for 0-1 and 1-2 hours respectively, (Fig 3.2.3). The mean increase in clearance of 10 % was significant ($p<0.05$) over time, and declining PAH plasma concentrations

COMPARISON BETWEEN THE CLEARANCES OF PAH AND AcPAH

The renal clearance of PAH fell from 0-1 to 1-2 hours whilst the renal clearance of AcPAH rose. Over both time periods, the PAH renal clearance was significantly lower than the AcPAH renal clearance ($p<0.01$) (526 v 806 and 241 v 891 ml/min/1.73 m^2 respectively). Similarly the 0-1 hour total body clearance of PAH was significantly less than the 0-1 hour AcPAH renal clearance ($p<0.01$; 600 v 806 ml/min/

Fig 3.2.3

The mean renal clearances of PAH and AcPAH (0-1 & 1-2 h) and the total body clearance of PAH (0-1 & 0-2 h), following a single intravenous injection of PAH, in 26 healthy male subjects. Bars = SD.

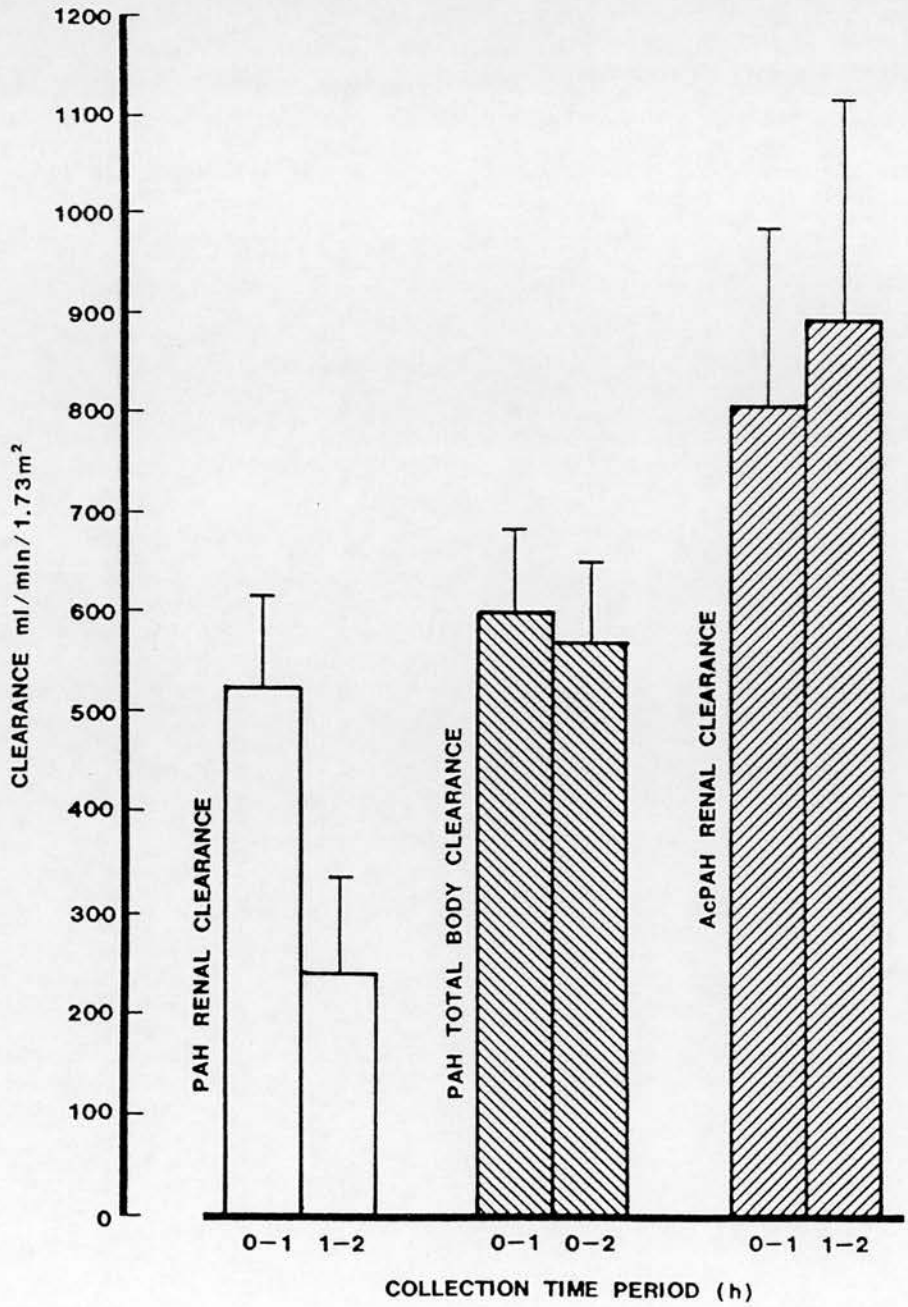


Fig 3.2.4

Relationship between the 0-1 h renal clearance of PAH and the corresponding 0-1 h total body clearance of PAH in 26 healthy males following a single intravenous injection of PAH.

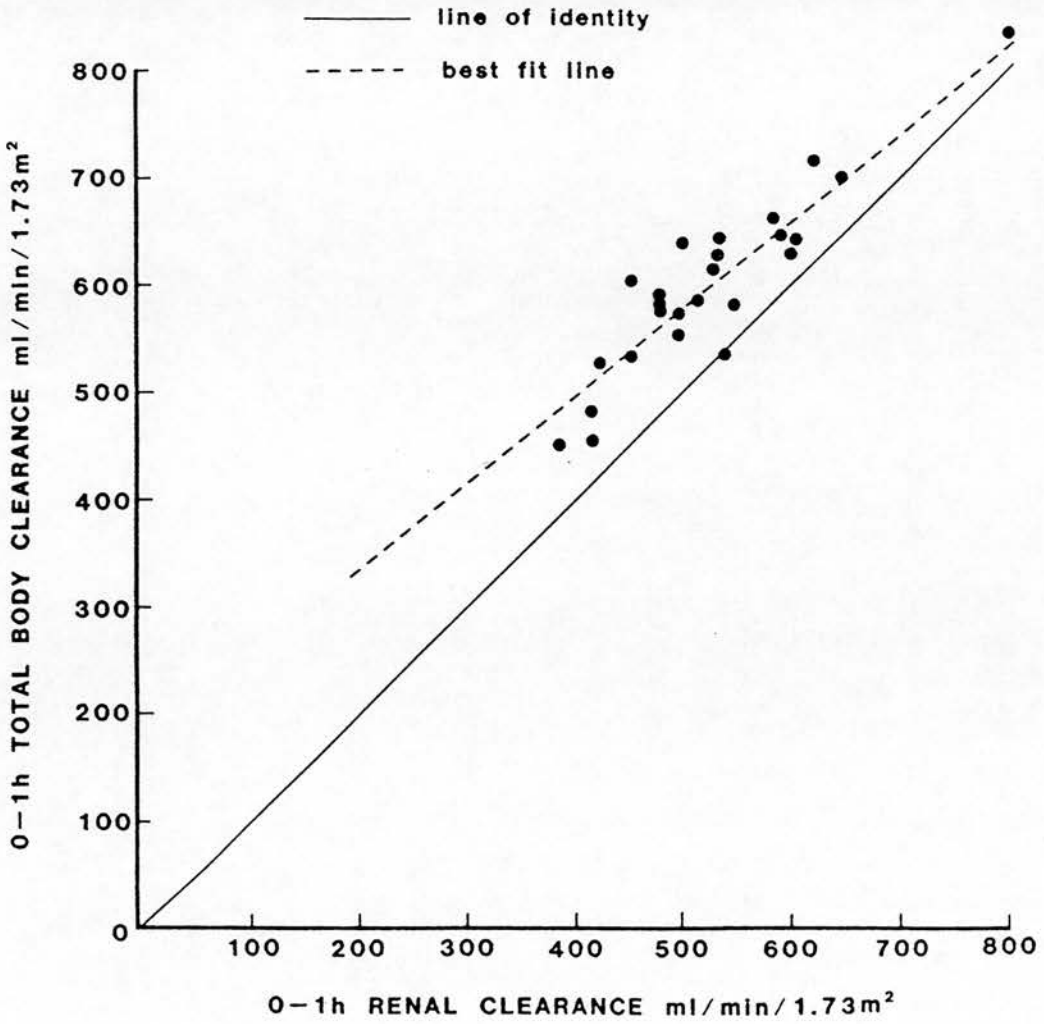


TABLE 3.2.2

The renal clearance of AcPAH (ml/min/1.73 m^2) following a single intravenous bolus injection of PAH in 26 healthy males. The elimination half life $t_{\frac{1}{2}}$ is also given.

	RENAL CLEARANCE (H)		
SUBJECT	0-1	1-2	$t_{\frac{1}{2}}$ (min)
GM	826	740	58.2
AT	874	1322	32.7
EC	734	890	41.4
WW	746	731	46.4
AB	722	927	38.2
RJ	983	996	44.0
JG	869	1259	37.7
AD	966	1074	34.8
AH	991	1258	35.1
SB	918	712	18.9
JA	806	905	41.7
PD	616	472	59.2
SA	1012	1141	39.2
RF	759	825	46.0
MS	832	754	50.9
PF	966	951	45.3
TM	595	730	49.6
MK	782	611	53.5
DM	1258	1290	58.1
JN	755	904	54.9
BH	601	750	45.4
BS	458	689	55.6
GS	913	863	55.6
LP	725	851	46.4
RM	417	624	40.2
CP	837	903	75.8
MEAN	806	891	48.0
$\pm$SD	181	223	10.2

1.73 m^2). A weak, but statistically significant correlation was found between the 0-1 hour renal clearances of PAH and AcPAH ($r=0.48$, $p<0.05$, Fig 3.2.5) but not between the 1-2 hour renal clearances.

Constant infusion

p-AMINOHIPPURIC ACID

The individual renal clearances of PAH in ten healthy male subjects for each collection period are given in Table 3.2.3.

The mean renal clearance of PAH for each urine collection period were 461, 445, 437 and 430 ml/min/1.73 m^2 for 1-1½, 1½-2, 2-2½ and 2½-3 hours respectively (Fig 3.2.6). The mean values show a tendancy for a fall in PAH clearance over time, but this was not statistically significant. The mean PAH clearance was 444 $\pm$ 66 ml/min/1.73 m^2.

ACETYL-p-AMINOHIPPURIC ACID

The individual renal clearances of AcPAH for the ten healthy male subjects for each collection period are given in Table 3.2.4.

The mean renal clearances of AcPAH were 566, 558, 563 and 589 ml/min/1.73 m^2 for 1-1½, 1½-2, 2-2½, and 2½-3 hours respectively (Fig 3.2.6). There was no statistically significant change over time, and the mean AcPAH clearance was 569 $\pm$ 108 ml/min/1.73 m^2.

COMPARISON BETWEEN THE RENAL CLEARANCE OF PAH AND AcPAH.

The PAH renal clearance was significantly lower than the corresponding AcPAH renal clearance on every occasion (Fig 3.2.6) and the overall difference was 22 % (444 v 569 ml/min/1.73 m^2; $p< 0.01$). There was no significant correlation between the renal clearances of PAH and AcPAH.

Fig 3.2.5

Relationship between the 0-1 h renal clearances of PAH and AcPAH following single intravenous administration of PAH in 26 healthy males.

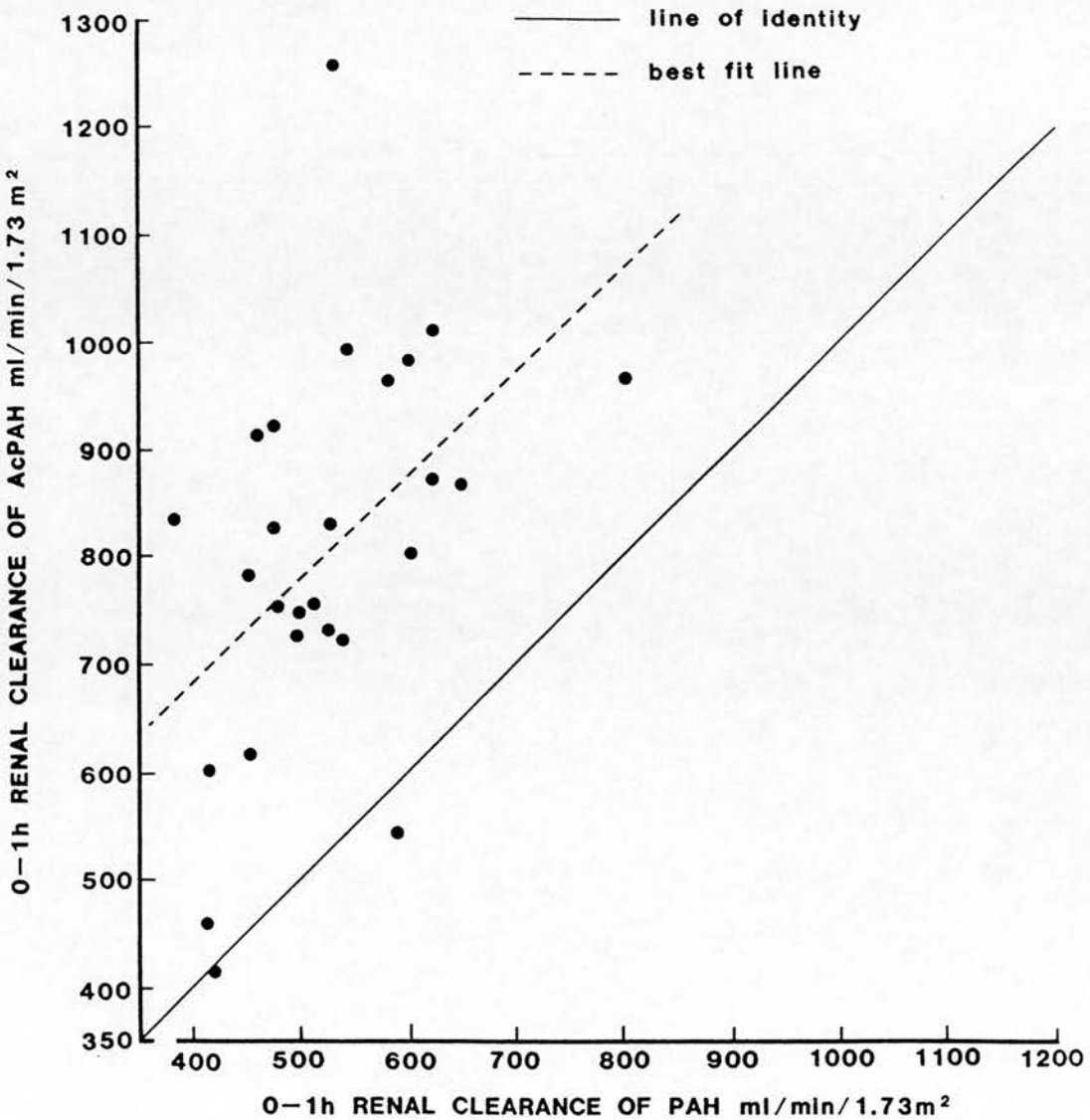


TABLE 3.2.3

The renal clearance of PAH (ml/min/1.73 m^2) during constant infusion of PAH in 10 healthy males.

	COLLECTION PERIOD (hours)					
SUBJECTS	1-1½	1½-2	2-2½	2½-3	Mean	±SD
MK	621	538	452	481	523	75
DM	509	480	514	474	494	20
JN	492	451	464	411	455	34
BH	395	482	465	419	440	40
BS	401	333	316	324	344	39
GS	327	416	371	393	377	38
LP	459	359	442	437	429	47
RM	480	500	513	496	497	14
CP	367	343	356	369	359	12
RJ	561	553	475	494	521	43
MEAN	461	445	437	430	444	
±SD	91	80	67	58	66	

Fig 3.2.6

Mean renal clearances of PAH and AcPAH for each urine collection period during a constant infusion of PAH in 10 healthy males. Bars = SD.

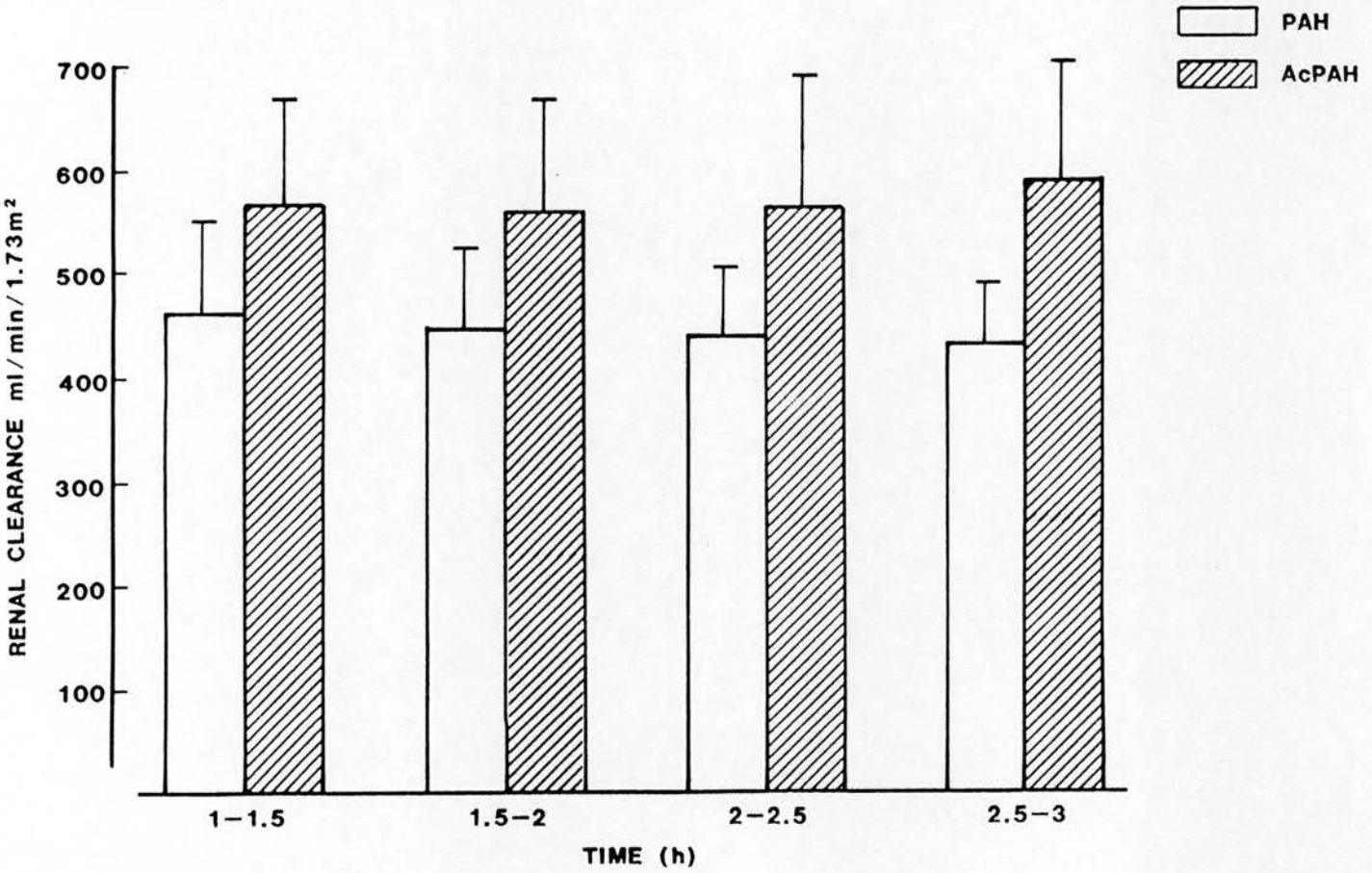


TABLE 3.2.4

The renal clearance of AcPAH (ml/min/1.73 m^2) during constant infusion of PAH in 10 healthy males.

	COLLECTION PERIOD (hours)					
SUBJECTS	1-1½	1½-2	2-2½	2½-3	Mean	±SD
MK	616	529	502	542	547	49
DM	657	683	748	778	717	56
JN	543	535	551	551	545	8
BH	433	510	485	494	481	33
BS	500	481	477	485	486	10
GS	632	610	591	556	597	32
LP	572	491	627	652	586	71
RM	478	451	366	518	453	64
CP	463	481	508	517	492	25
RJ	765	805	774	797	785	19
MEAN	566	558	563	589	569	
±SD	103	111	126	114	108	

The effect of urine flow rate on the renal clearance of PAH and AcPAH.

Single injection

The individual urine flow rates are given in Table 3.2.5.

The urine flow rate fell from 7.00 to 6.00 ml/min from 0-1 to 1-2 hours, but no significant correlation was found between urine flow rate and the renal clearances of PAH or AcPAH.

Constant infusion

The individual urine flow rates are given in Table 3.2.6.

The mean urine flow rates were similar, over all collection periods (6.7, 6.4, 6.0, and 6.0 ml/min for 1-1½, 1½-2, 2-2½, and 2½-3 hours respectively). There was no correlation between urine flow rate and PAH or AcPAH clearances.

Urinary recovery

Single injection

The individual urinary recoveries of PAH and AcPAH are given in Tables 3.2.7 and 3.2.8 respectively.

The mean urinary recovery of the administered dose of PAH in the first four hours was 82.4 ± 6 % (range 75-92 %) (Fig 3.2.7) and 92 % of this was excreted in the first hour.

The mean urinary recovery of AcPAH over 8 hours was 17.1 ± 2 % (range 13-23 %) (Fig 3.2.7), and 51 % of this was excreted in the first hour.

Constant infusion

The mean urinary recoveries of PAH and AcPAH in each urine collection period expressed as a percentage of total PAH + AcPAH were 89.5 % for PAH, and 10.5 % as AcPAH.

TABLE 3.2.5

Urine flow rate (ml/min) following a single intravenous bolus injection of PAH in 26 healthy males.

	COLLECTION PERIOD (h)	
SUBJECT	0-1	1-2
GM	1.5	9.8
AT	6.0	4.8
EC	8.4	5.8
WW	8.4	4.3
AB	10.9	5.5
RJ	7.7	6.8
JG	9.3	7.0
AD	7.5	4.8
AH	6.6	7.2
SB	2.0	2.6
JA	6.7	4.6
PD	8.4	4.8
SA	9.6	5.2
RF	4.3	8.5
MS	7.9	5.6
PF	12.5	5.5
TM	8.6	5.1
MK	*	7.1
DM	1.6	5.7
JN	*	6.1
BH	7.5	7.2
BS	10.7	6.3
GS	*	9.3
LP	*	7.1
RM	*	5.8
CP	0.6	1.7
MEAN	7.0	6.0
±SD	3.3	1.8

* = Urine collection period not timed

TABLE 3.2.6

Urine flow rate (ml/min) during constant infusion of PAH in 10 healthy males

	COLLECTION PERIOD (h)					
SUBJECTS	1-1½	1½-2	2-2½	2½-3	Mean	±SD
MK	9.3	9.0	4.5	8.2	7.8	2.2
DM	7.6	6.6	4.0	7.1	6.3	1.6
JN	7.7	4.3	3.8	5.0	5.2	1.7
BH	4.9	4.9	6.5	5.5	5.4	0.7
BS	5.8	3.6	6.1	7.0	5.6	1.4
GS	7.0	8.1	4.4	4.5	6.0	1.8
LP	9.6	8.8	4.5	4.8	6.9	2.6
RM	7.8	7.0	11.7	8.5	8.8	2.0
CP	2.3	7.0	7.0	7.1	5.8	2.4
RJ	4.6	4.2	7.5	2.6	4.7	2.0
MEAN	6.7	6.4	6.0	6.0	6.3	
±SD	2.3	2.0	2.4	1.9	1.2	

TABLE 3.2.7

The urinary recovery of PAH (% of dose) following a single intravenous bolus injection of PAH in 26 healthy males.

	Collection periods (h)				
SUBJECT	0-1	1-2	2-3	3-4	Total (0-4)
GM	72.4	6.4	1.5	0.7	81
AT	76.9	4.0	0.8	0.5	82
EC	76.4	3.7	1.1	0.6	81
WW	80.0	3.1	1.1	0	84
AB	86.4	3.4	1.2	0.7	92
RJ	85.5	2.7	0.7	0.4	89
JG	83.9	3.2	0.8	0.6	88
AD	89.7	2.2	0.7	0.4	93
AH	83.4	3.6	0.8	0.6	88
SB	76.1	3.1	1.0	0.3	80
JA	84.6	4.2	1.2	0.4	90
PD	73.2	3.6	0.8	0.9	79
SA	85.3	3.8	0.9	0.3	90
RF	74.7	4.1	0.8	0	78
MS	73.2	3.3	0.6	0.1	77
PF	81.4	2.5	0.4	0.1	84
TM	74.5	3.8	0.8	0.3	86
MK	66.8	10.7	2.2	0	80
DM	70.3	5.0	1.4	0.6	77
JN	69.6	4.9	0.9	0.4	76
BH	70.7	6.2	1.0	0.4	78
BS	70.3	5.9	1.1	0.5	78
GS	71.1	6.3	0.6	0	78
LP	69.4	4.9	0.8	0.2	75
RM	70.8	3.0	0.6	0.2	75
CP	72.4	5.9	1.2	0.1	80
MEAN	76.5	4.4	1.0	0.3	82
±SD	6.5	1.8	0.4	0.3	6

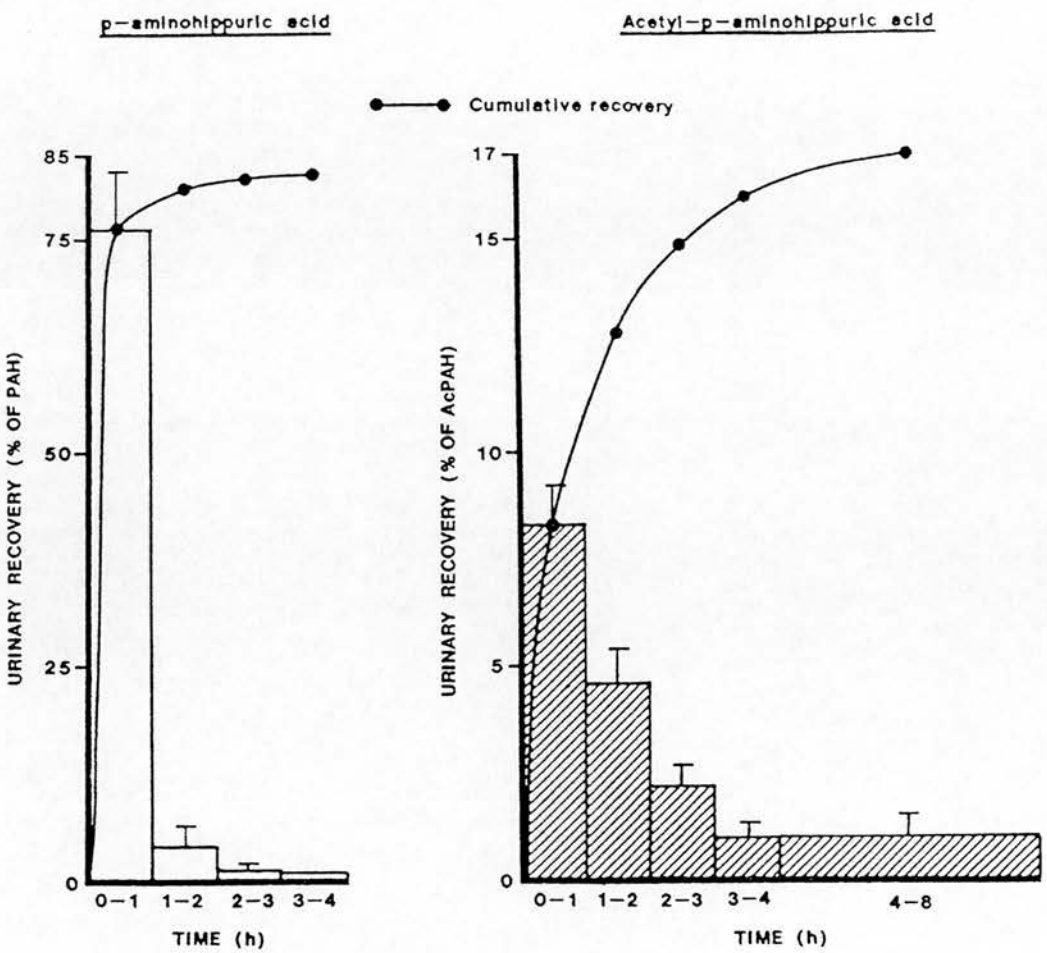
TABLE 3.2.8

The urinary recovery of AcPAH (% of dose of PAH) following a single intravenous injection of PAH in 26 healthy males.

	Collection period (h)					
SUBJECT	0-1	1-2	2-3	3-4	4-8	total(0-8)
GM	6.1	4.3	2.1	1.3	1.6	15.4
AT	8.8	5.2	1.4	0.6	0.4	16.4
EC	8.6	4.2	2.0	1.0	1.2	17.1
WW	9.8	5.0	2.2	1.0	1.0	19.0
AB	7.6	3.7	1.8	0.8	0.8	14.7
RJ	9.6	4.7	2.0	1.0	0.8	18.2
JG	8.3	4.8	2.0	0.8	0.6	16.4
AD	9.1	3.5	1.4	0.6	0	14.6
AH	8.5	4.4	2.1	0.9	1.0	16.9
SB	9.9	4.6	2.1	1.1	1.3	18.9
JA	6.9	3.6	1.5	0.7	0.4	13.0
PD	8.5	4.0	3.7	1.5	1.4	19.0
SA	9.0	4.7	2.4	0.8	1.0	17.9
RF	8.8	4.6	2.1	1.1	1.4	18.0
MS	8.0	3.9	2.0	1.0	1.2	16.1
PF	7.7	3.1	1.4	0.7	0.5	13.4
TM	7.3	4.6	1.8	1.1	1.0	15.8
MK	7.5	4.6	2.5	0.9	1.0	16.5
DM	9.4	5.9	2.6	1.1	1.0	20.0
JN	8.2	5.8	2.5	1.4	1.9	19.7
BH	6.9	4.4	2.0	1.1	1.3	15.8
BS	6.9	5.7	2.5	1.2	1.5	17.8
GS	8.0	4.7	2.3	1.2	1.3	17.5
LP	8.8	4.9	2.5	1.0	1.6	18.7
RM	6.9	3.9	1.7	1.1	0.7	14.3
CP	8.8	7.1	3.9	1.9	1.6	23.3
MEAN	8.2	4.6	2.2	1.0	1.1	17.1
±SD	1.0	0.8	0.6	0.3	0.5	2.3

Fig 3.2.7

The mean urinary recovery of PAH and AcPAH, (percentage of the PAH dose) for each urine collection period, following a single intravenous injection of PAH in 26 healthy males. Bars = SD.



Comparison of clearances in 10 healthy males who underwent both constant infusion and single injection of PAH

The individual clearance data for PAH and AcPAH are given in Table 3.2.9.

p-Aminohippuric acid

The mean 0-1 hour renal clearance of PAH following a single injection was similar to that determined during constant infusion (465 $\pm$ 65 vs 444 $\pm$ 65 ml/ min/1.73 m^2), and the difference between these values was not statistically significant. However, the mean 1-2 hour renal clearance of PAH after single injection was significantly lower than the mean clearance determined during constant infusion (274 v 444 ml/min/1.73 m^2; $p< 0.01$) (Fig 3.2.8). The 0-1 hour total body clearance of PAH overestimated the renal clearance of PAH, determined during constant infusion, by 20.6 % (559 vs 444 ml/min/ 1.73 m^2; $p<0.01$). There was a weak, but significant correlation between the total body clearance of PAH following single injection, and the renal clearance during constant infusion ($r= 0.65$, $p<0.05$, Fig 3.2.9), but not between the renal clearance determined by the two methods.

Acetyl-p-aminohippuric acid

The clearance of AcPAH following single injection of PAH was significantly greater than during constant infusion of PAH, ($p< 0.01$), 773 and 848 ml/min/1.73m^2 for the 0-1 and 1-2 hour periods after single injection, compared with 569 ml/min/1.73m^2 during the constant infusion (Fig 3.2.8). There were significant correlations between the renal clearances following single injection, and constant infusion for both 0-1 hour ($r= 0.817$, $p<0.01$, Fig 3.2.10a) and 1-2 hour ($r= 0.743$, $p<0.05$, Fig 3.2.10b) respectively.

TABLE 3.2.9

The clearances of PAH and AcPAH (ml/min/1.73 m^2) during constant infusion and following a single injection of PAH in 10 healthy males.

	CONSTANT INFUSION		SINGLE INJECTION				
			Renal Cl		Total Cl	Renal Cl	
	PAH	AcPAH	PAH	PAH	PAH	AcPAH	AcPAH
SUBJECTS	mean	mean	0-1	1-2	0-1	0-1	1-2
MK	523	547	451	599	602	782	611
DM	494	717	532	277	642	1258	1290
JN	455	545	478	210	585	755	904
BH	440	481	414	337	482	601	750
BS	344	486	415	262	456	458	689
GS	377	597	460	305	573	913	863
LP	429	586	497	255	640	725	851
RM	497	453	420	122	526	417	624
CP	359	492	384	178	451	837	903
RJ	521	785	600	196	631	983	996
MEAN	444	569	465	274	559	773	848
±SD	66	108	65	131	75	250	201

Fig 3.2.8

The mean renal clearances of PAH and AcPAH following a single injection and during constant infusion of PAH, in 10 healthy males. The total body clearance of PAH following a single injection of PAH in these subjects is also shown. Bars = SD.

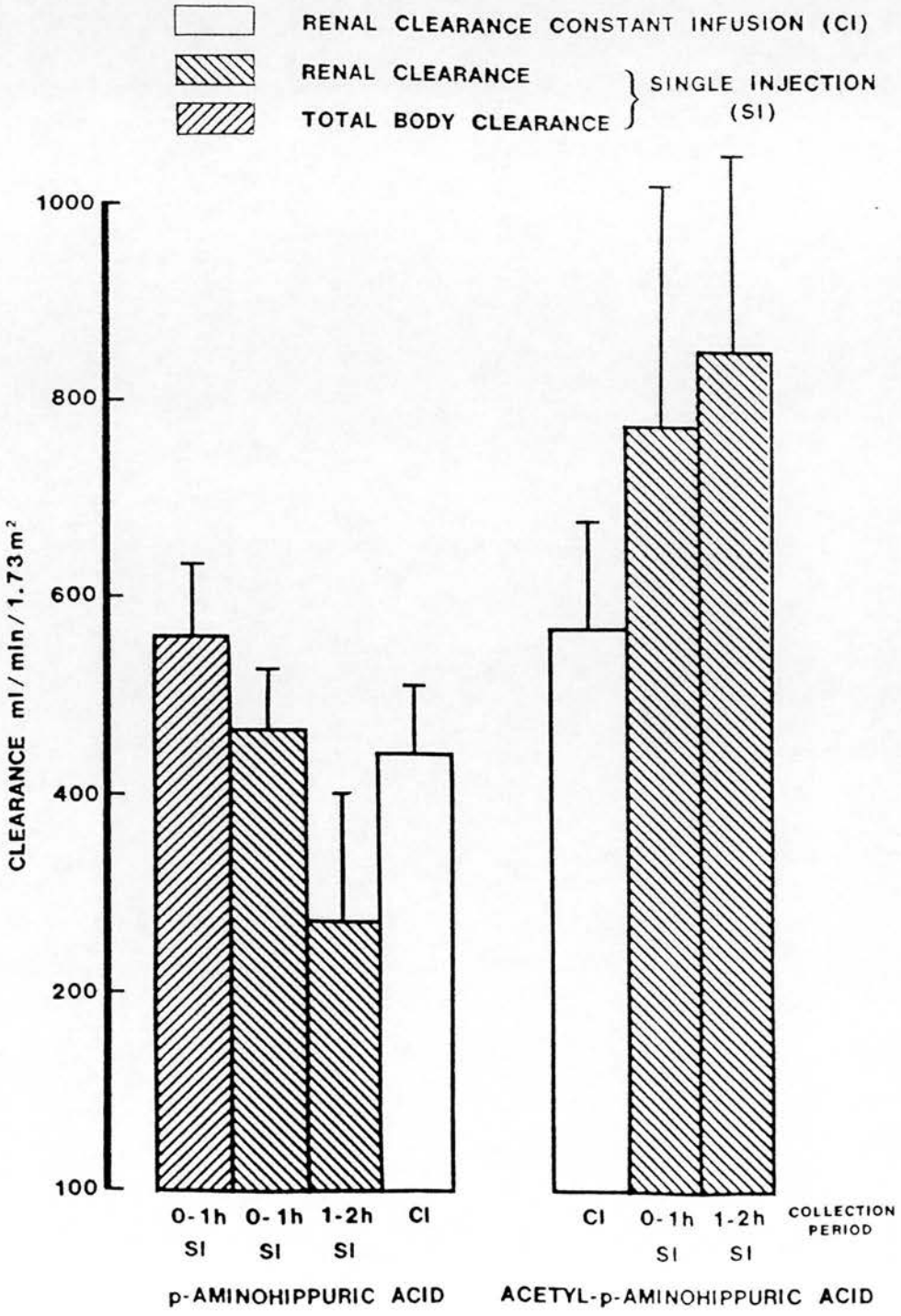


Fig 3.2.9

The relationship between the mean renal clearance of PAH during constant infusion and the 0-1 h total body clearance of PAH following a single intravenous injection of PAH in 10 healthy males.

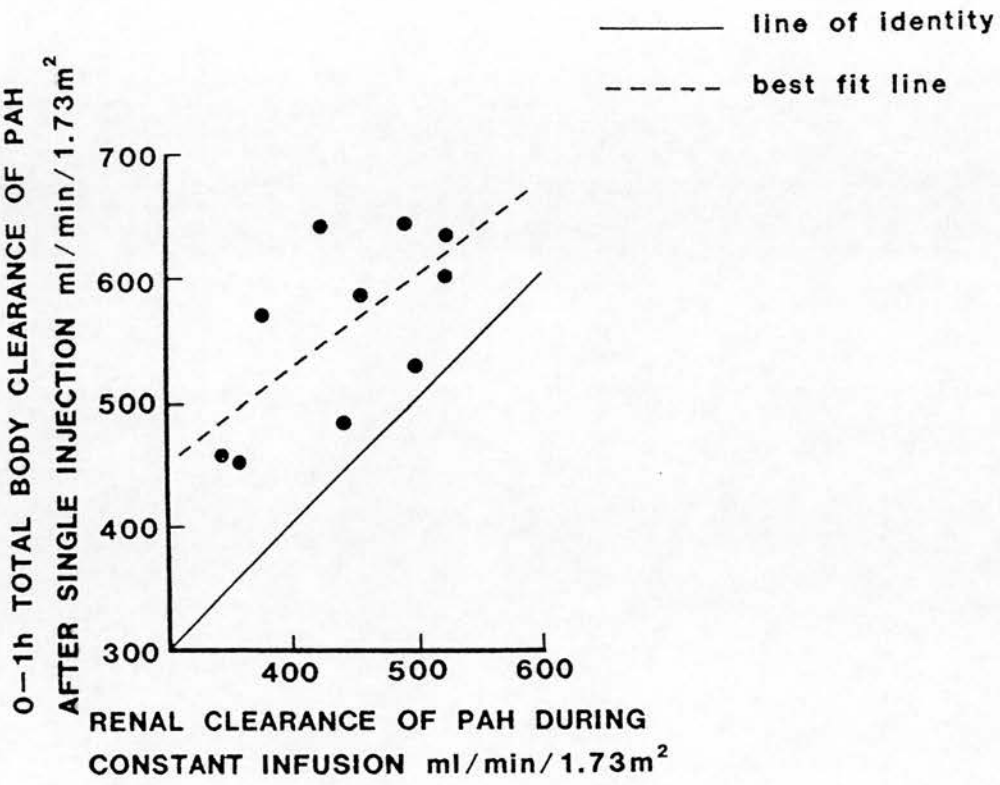
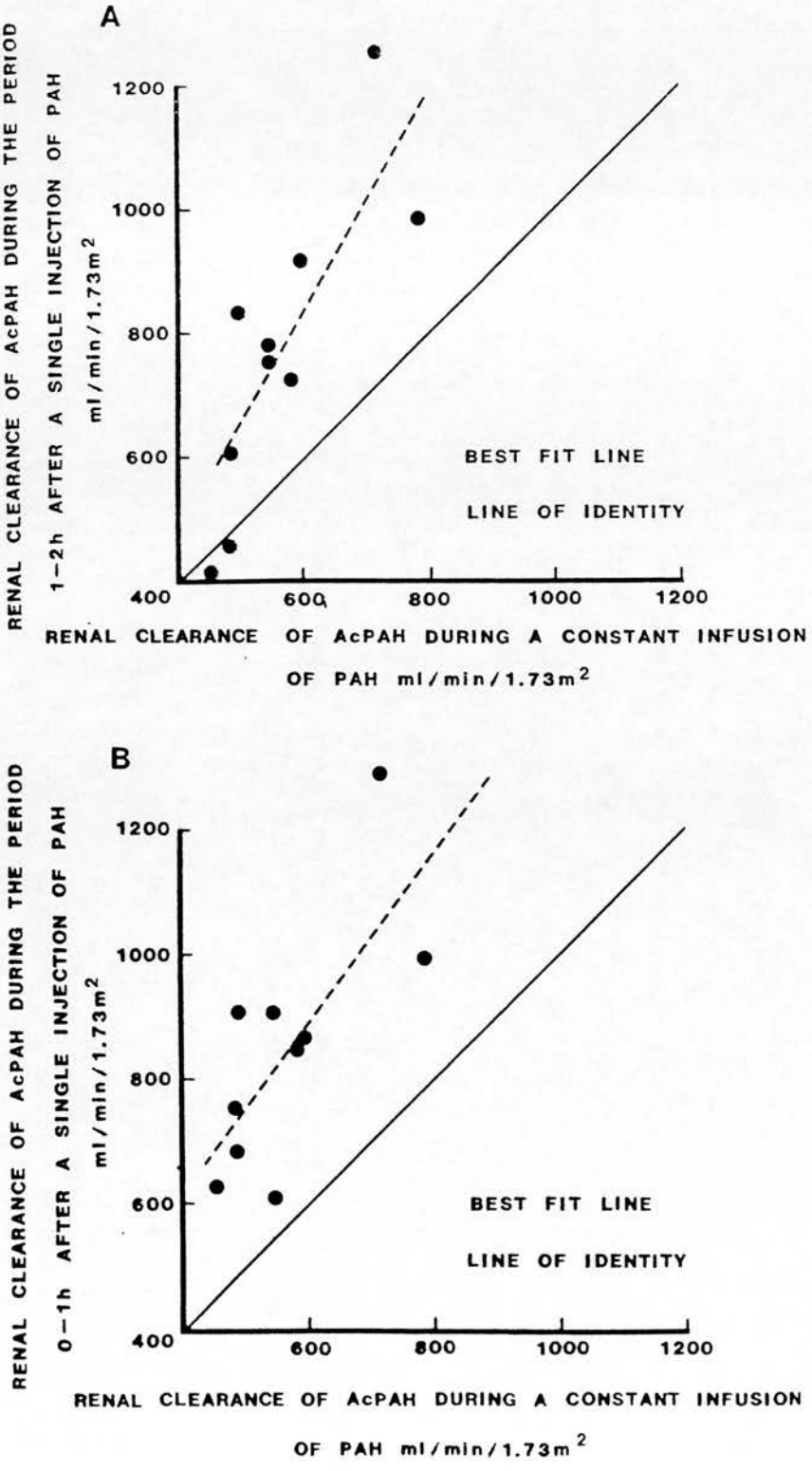


Fig 3.2.10

Relationship between the renal clearances of AcPAH (0-1 h (A) and 1-2 h (B)) after a single injection and during a constant infusion of PAH in 10 healthy males.



DISCUSSION

The acetylation of p-aminohippuric acid (PAH) is known to occur in animals and man, but investigations in man are limited, and the assay unreliable. About 20 % of a dose of PAH is converted to AcPAH and, as a result, simple methods for estimating PAH by single injection and constant infusion without urine collection, give a substantial overestimate of the renal clearance. In the present study, the disposition and kinetics of PAH and AcPAH have been investigated in healthy male subjects, using specific assays for PAH and AcPAH.

Following single intravenous injection of PAH, plasma concentrations initially fall rapidly, followed by a slower decreasing rate of decline. The transition between rapid and slow phases occurred after 20 to 30 minutes, and the distribution rate constant calculated by 2 compartment pharmacokinetic analysis indicated greater than 95 % distribution in 30 minutes. However, plasma concentrations and the rate of elimination of PAH decreased progressively over time, reflecting the progressive fall in its renal clearance. The plasma concentration time data could be fitted to a two compartment model over the first hour, and the total body clearance was calculated on the assumption that the clearance during this period, was maintained. In previous studies, the distribution of PAH following a single injection was reported to be complete within 20 to 50 minutes (Tacket & Houck, 1950; Rosenbaum et al, 1973). These authors reported that the plasma concentrations of PAH declined in a linear fashion after distribution, but others report a linear decline almost immediately following the intravenous bolus injection (Newman et al, 1949). The striking difference in the disposition of PAH observed in the present study, can probably be accounted for by infrequent sampling in the early studies. In the present study, numerous samples

were taken to properly define the plasma decay curve of PAH. The plasma concentration-time decay curve of PAH has been reported to be consistent with an open two compartment pharmacokinetic model in the dog (Mandel et al, 1955), but appropriate pharmacokinetic modelling of PAH does not seem to have been reported in man. The mean plasma half life of PAH estimated from the 0-1 hour plasma data was 22 minutes, and this is double that reported by Moffat et al, 1986.

AcPAH was detected in the plasma 3 minutes after a single injection of PAH, and the plasma concentration of this metabolite rose slowly to reach a maximum at 20 minutes, before declining in a linear fashion. The rate of elimination of PAH was more than double that of AcPAH, and since the renal clearance of AcPAH is greater than that of PAH, this implies that AcPAH has a larger volume of distribution. Plasma concentrations of AcPAH following a single intravenous injection of PAH have not been reported previously in subjects with normal renal function, although Newman et al, (1949) estimated that the renal clearance of AcPAH was 800 ml/min, following a single injection of PAH. Statius Van Eps et al, (1967) described a rapid decline in plasma concentrations of PAH, followed by a slow appearance of AcPAH with the plasma concentrations peaking around 10 hours, following a single injection of PAH in patients with renal impairment.

During constant infusion of PAH, both PAH and AcPAH plasma concentrations appeared to reach steady state, but the latter were an order of magnitude less than those of the parent compound. Plasma concentrations of AcPAH during constant infusion of PAH, have not been reported in subjects with normal renal function, due to low levels (Brown et al, 1974). High concentrations of AcPAH have been detected in patients with renal impairment, and renal artery stenosis (Grindt et al,

1974). These authors also measured renal venous blood, and found that p-aminobenzoic acid and acetyl p-aminobenzoic acid concentrations were greater, than in mixed venous blood. The method of assay used by these workers was not specific. P-aminobenzoic acid was not detected in the present study.

Following a single intravenous injection, PAH was rapidly excreted into the urine, as would be expected of a substance cleared at the rate of the renal blood flow, but only 82 % of the administered dose was recovered in the urine as PAH. Most was excreted in the first hour, and elimination was essentially complete by four hours. AcPAH could be measured in the urine up to eight hours, and 17 % of the administered dose of PAH was recovered as AcPAH. Virtually the total dose of PAH was recovered as PAH plus AcPAH, suggesting that, renal excretion is the sole route of elimination of PAH and AcPAH. Under the conditions of this study, AcPAH appeared to be the sole metabolite of PAH.

The renal clearance of PAH is universally accepted as a reliable estimate of the renal plasma flow but, it is only 90 % extracted in one passage through the kidney, and the term effective renal plasma flow (ERPF), should be used (Smith, 1951). As the extraction ratio and haematocrit vary between individuals, the clearance values in this study are taken to represent ERPF. As PAH is actively secreted by a specific proximal tubular transport system, its extraction decreases at high plasma concentrations, because of saturation (Weiner, 1985). Depression of the PAH clearance occurs at plasma concentrations above 80 mg/l in healthy subjects, with complete saturation occurring above 100 mg/l (Schuster & Seldin, 1985). The plasma concentrations of PAH in the present study were about 30 mg/l, during constant infusion, which is within the recommended range for maximal extraction of PAH and thus, maximal and constant

clearance (Smith, 1951). Following single intravenous bolus administration, the maximum plasma concentrations of PAH were about 60 to 70 mg/l, but these fell rapidly to less than 30 mg/l by 15 minutes. The mean midpoint plasma concentration during the first hour was 15 mg/l, this is well below those achieved during the constant infusion. Thus, after a single intravenous injection, the concentrations of PAH were in the range where the clearance of PAH should have been maximal and constant.

Following a single intravenous injection, the renal clearance of PAH showed a highly significant fall over time and plasma concentration. The renal clearance during the second hour were only about 54% of that during the first hour. This dramatic fall in PAH clearance was surprising, as by all previous accounts the PAH plasma concentrations were well below the levels associated with saturation of active transport, and constant maximal clearances were expected. During the 1-2 hour period, the mean plasma concentrations of PAH were less than 7 mg/l, and the PAH clearance was grossly reduced and, not constant. The 0-1 hour total body clearance of PAH was significantly greater than the renal clearance of PAH by 14 %, and this can be accounted for by the metabolism of PAH to acetyl PAH. The dramatic fall in PAH clearance following single intravenous injection is not consistent with the findings of Landowne & Alving, (1947) and Tacket & Houck, (1950). These investigators did not observe a fall in the renal clearance of PAH following a single injection and indeed, the latter even reported a rise in the renal clearance of PAH, with time. Tacket & Houck, also compared the total body and renal clearances of PAH. However, they used an inappropriate one compartment model. They could only obtain constant results by using the volume of distribution of mannitol, and carrying out hydrolysis of the plasma and urine samples. Newman et

al, (1949) reported a fall in the renal clearance of PAH in man, but not in dogs. In these reports, the renal clearance of PAH was estimated by dividing the amount excreted by the midpoint plasma concentration, taken from a semilogarithmic plot against time. This is not correct because the distribution of PAH is ignored (Notari, 1987). Newman et al,(1949), also reported that the clearance of AcPAH was greater than the PAH clearance, following a single injection of PAH. They calculated the clearance of AcPAH by estimating the difference in PAH content of plasma and urine before, and after hydrolysis. This indirect method of estimating AcPAH is subject to interference from endogenous substances, which also give a positive reaction (Brown et al, 1976). Despite this, their estimate of the AcPAH clearance after PAH administration was similar to that found in the present study.

The mechanism of this striking fall in the PAH clearance is unknown, and may be due to factors such as time, arterial-venous plasma concentration differences, delay time or urine flow dependant clearance. However, no correlation between urine flow and clearance of PAH and AcPAH was found in the present study, and a time effect also seems unlikely as the fall in the clearance of PAH during the constant infusion studies was minor, and can probably be attributed to failure to reach steady state conditions.

Arterial-venous differences and delay time may cause errors, when plasma concentrations are rapidly decreasing (Brun et al, 1949). Arterial plasma concentrations were not estimated in this study but, in previous literature reports, the mean venous renal clearance of PAH was only 16 % less than the arterial clearance (Tacket & Houck, 1950). Similar findings were reported by Newman et al, (1949), but they also showed that the arterial renal clearance of PAH fell in the

same manner as the venous clearance, over two hours. Higher venous than arterial concentrations are unlikely to account completely for the low clearance. Delay time can also cause significant errors because the excretion rate lags behind the plasma concentration. In the present study, long collection periods and water diuresis were used to reduce this error. Using the formula of Nosslin, (1965) the correction time in the first hour with a mean urine flow rate of 7 ml/min, was 4 minutes. In 60 minutes this represents an error of 6.6 % and, as the subjects voided urine at least two minutes after the plasma samples were taken, this error was reduced to only 3 %. Similar errors could be anticipated for the second hour, and this therefore, cannot explain the fall in PAH clearance.

The fall in PAH clearance was quite clearly plasma concentration-dependant. However, it is generally believed that PAH clearance is maximal and constant, at low plasma concentrations. In the present study, the plasma concentrations of PAH following a single injection, were much lower in the second hour than in any previous investigation, and at these levels the PAH clearance was highly dependant on plasma concentrations. One possible explanation for this could be acetylation of PAH within the kidney. This would also explain the significantly higher clearance of AcPAH, than that of the parent compound. Newman et al, (1949), suggested that the declining renal clearance of PAH was due to renal conjugation to the acetyl derivative but, Mandel et al, (1955) attributed the fall seen by these authors to inappropriate kinetic analysis. In the present study, the amount of conjugated PAH in the urine increased over time. However, an increase in the urinary excretion of acetyl PAH with time, would in any event be expected since its formation is delayed, and its half-life was much longer than that of PAH. Other possible factors

include a decrease in renal extraction, due to increased plasma protein binding. This has been reported for other compounds used for measuring renal blood flow such as, diodrast (Block & Burrows, 1960). Increased uptake into red blood cells at low concentrations or renal tubular competition, could also account for a reduced extraction. One other strong possibility is tubular reabsorption of PAH, as reported to occur at low plasma concentrations in dogs (Cho & Cafruny, 1970). These possible mechanisms for the decline in PAH clearance are discussed, in more detail, in the next section.

Comparison of the clearance of PAH using the single injection and constant infusion methods in the same 10 individuals, gave virtually identical results for the mean values but, there was no significant correlation between the individual clearances for the two methods. During the first hour after single intravenous administration, the mean total body clearance of PAH consistently overestimated the renal clearance during constant infusion by 20 %, and a similar discrepancy has been observed by others in man (Rosenbaum et al, 1973; Boineau et al, 1974), but not in dogs (Mandel et al, 1955). This is not surprising, as dogs cannot acetylate drugs (Newman et al, 1949). The renal clearance of AcPAH was significantly greater following a single injection of PAH than during constant infusion, and this would be consistent with some renal acetylation of PAH which is greater at low rather than high concentrations of PAH, since concentrations were lower on average, following a single injection than during constant infusion. This is discussed in more detail in section 3.

The use of the single injection method with PAH for measuring the renal plasma flow seems, on the face of it, to be limited. The renal clearance of PAH falls progressively as plasma concentrations decline, and the total body clearance is an overestimate due to

metabolism. However, the renal clearance of PAH determined by the single injection method over the first hour gives the same results, as that found with the constant infusion method. It can therefore be used as a quick, simple measure of the PAH clearance, and repeated measurements could be made on the same day. The major disadvantage is that urine collections are still needed. Although errors are involved in urine collection (Zender et al, 1968), it may only be necessary to determine the fractional conversion to AcPAH, if the administered dose is known. However, this assumes that PAH is not acetylated in the kidney and the total body clearance could then be corrected for the fraction metabolised. There is a good correlation between the renal clearance and total body clearance of PAH, but a simple universal correction factor cannot be used, because of individual differences. There is also more extensive and variable acetylation of PAH in patients with impaired renal function (Statius Van Eps et al, 1967). In addition acetylation of some compounds are genetically controlled and the population can be divided into fast and slow metabolisers (Weber & Hein, 1985). In the present study, there was no evidence of separate groups of slow and fast acetylators, but formal testing of acetylator status in the volunteers, was not carried out.

SUMMARY

The disposition and kinetics of PAH and AcPAH have been investigated following intravenous administration of PAH by constant infusion, and single rapid injection. Following a single injection of PAH, a mean of 82 % of the dose administered was recovered in the urine as PAH and the remainder, as AcPAH. The total urinary recovery as these two compounds, was essentially complete. The total body clearance of PAH was significantly greater than the renal clearance, and this can be attributed to the acetylation of PAH. The renal clearances of PAH

following a single injection fell dramatically over time, as plasma concentrations decreased after the first hour. The clearance of PAH appears to be plasma concentration dependant, when these are low. AcPAH appears to be the sole metabolite of PAH, and its clearance is significantly greater than that of PAH during constant infusion and, following single intravenous administration of PAH. Possible explanations for these results are a reduced extraction of PAH compared to AcPAH, saturable tubular reabsorption of PAH, or PAH is metabolised in part within the kidney.

The renal clearance of PAH during the first hour following a single intravenous injection was similar to that during constant infusion, and the method can be used as an estimate of ERPF, as long as urine is collected.

SECTION III

THE PLASMA CONCENTRATION DEPENDANT CLEARANCE OF p-AMINOHIPPURIC ACID

INTRODUCTION

In the last section the disposition and kinetics of p-aminohippuric acid (PAH) were investigated, and it was found that 17 % of the dose following single injection was metabolised to acetyl-p-aminohippuric acid (AcPAH). The total body and renal clearances of PAH fell dramatically after the first hour, whilst the AcPAH renal clearances rose. The plasma concentrations of PAH achieved after the single injection were well below those at which tubular transport is saturable and therefore, the clearance of PAH should be independent of the plasma concentration (Smith, 1951). This depression in the renal clearance of PAH has been reported previously by Newman et al, (1949). Smith (1951), also cited Hamburger and Ryckewaert, who observed very low PAH clearance at plasma concentrations below 10 mg/l after a single injection and during constant infusion. These authors suggest that this may be due to tubular conjugation or high plasma protein binding of PAH, or tubular "inertia". Smith (1951), argued against significant renal metabolism at low PAH plasma concentrations. Others have not reported any decline in PAH clearance after administration of PAH by single injection (Landowne & Alving, 1947; Tacket & Houck, 1950). However, the PAH clearance may become dependant on its plasma concentration at low concentrations.

The object of this study was to investigate the relationship between clearances of PAH and AcPAH at different steady state plasma concentrations of PAH, to the plasma concentrations of PAH. PAH plasma concentrations were therefore incrementally increased and decreased, in a steplike manner.

METHODS

8 healthy male volunteers mean age 30 $\pm$ 6 years (range 25-42 yrs) weighing 55-77 Kg (mean 69 $\pm$ 7 Kg)

received two constant infusions of PAH in random order on different days. The constant infusions were intended to give a stepwise increase in plasma concentrations of PAH on one occasion, and a stepwise decrease on the other. The interval between the two studies ranged between 7 and 22 days in seven subjects, and six months in the other. The same batch of sodium aminohippurate (20 % w/v, Merck Sharp and Dohme, U.K.) was used throughout.

The procedure was as described previously in Chapter two, section III (p 89) for the infusion of inulin. On the "step up" study days, the subjects were given loading doses of sodium aminohippurate of 200 mg (1 ml), 200 mg (1 ml) and 400 mg (2 ml) over 1 minute, prior to the increase in the infusion rate for the low, mid and high periods respectively. The infusion fluid contained sodium aminohippurate (12.67 mg/ml in 300 ml of 0.9 % saline) and the low, mid and high dose rates were 2.5, 5 and 15 mg/min respective. On "step down" study days, the subjects received an initial loading dose of sodium aminohippurate (500 mg, 2.5 ml) over 1 minute, followed by infusion of 14.4, 4.8 and 2.4 mg/min for the high, mid and low periods respectively. The times of urine collection, blood sampling and changes in the infusion rate for both step up and down studies were the same as for inulin (p 89) (Fig 3.3.1). Ten ml of the infused solution was collected at the end of each study for measurement of the PAH concentration.

Sample collection

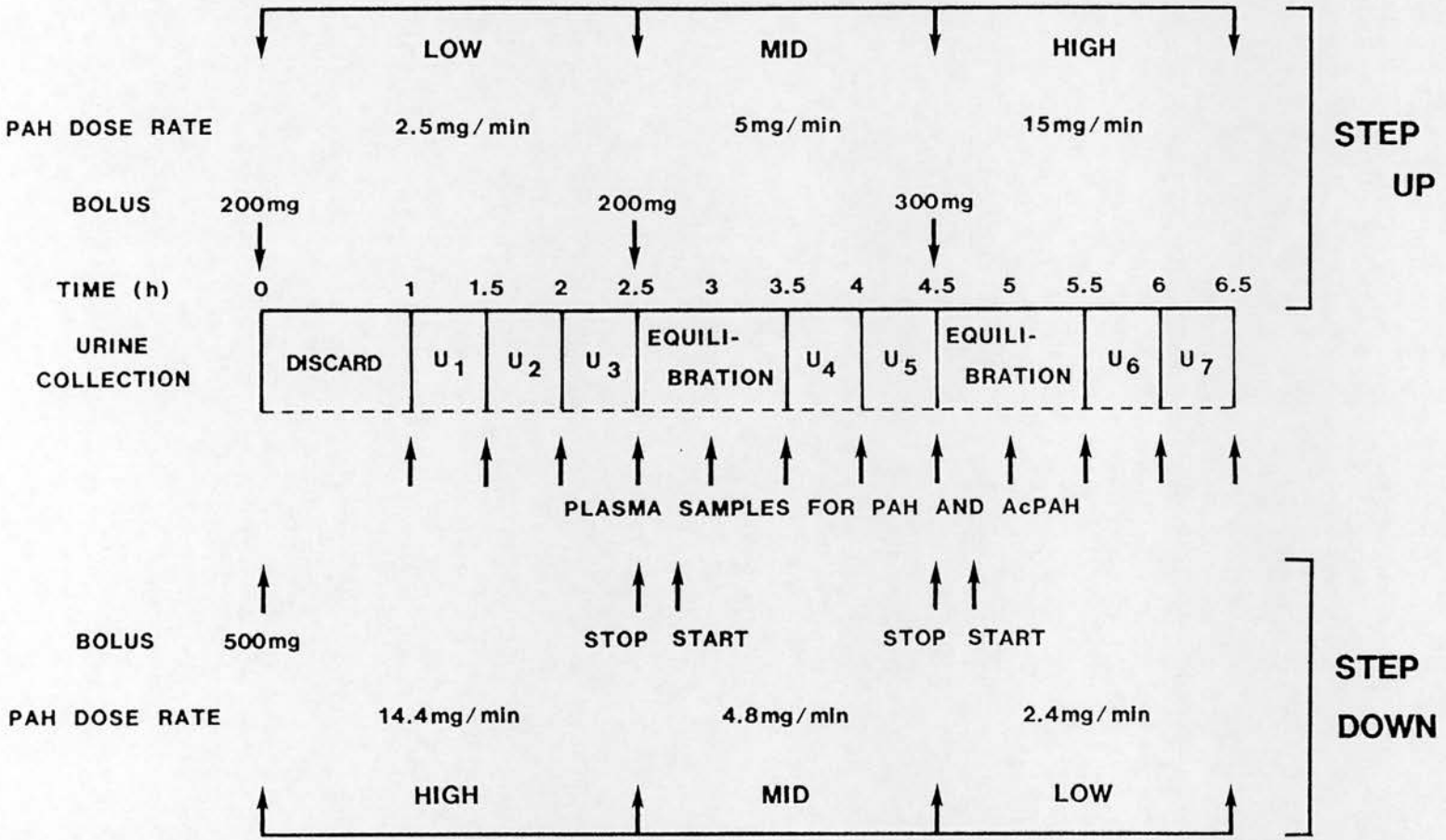
Plasma and urine samples were collected and analysed for PAH and AcPAH as described in section II (p 174).

Data analysis

Because of time needed for equilibration after changing the rate of administration of PAH, the data for

Fig 3.3.1

Plan of step up and step down constant infusion studies with PAH



the first urine collection period after each dose change (U1, U4, U5, U8, and U9, Fig 3.3.1) have been disregarded. The samples were pooled, to represent the periods from 1½ to 2½, 3½ to 4½ and 5½ to 6½ hours. In the "step up" study, these successive periods are referred to as "low", "mid", and "high" dose infusions and the reverse applies for the "step down" study (Fig 3.3.1).

The renal clearances of PAH and AcPAH were calculated, as described previously for the constant infusion method (chapter 2, p 92).

The total body clearance of PAH (TBC) was calculated from the following relationship :-

$$TBC = \frac{\text{rate of infusion}}{Css} \qquad \text{(Equation 5)}$$

where Css is the steady state plasma concentration of PAH calculated as the mean of the last two determinations of the plasma PAH concentration, during each infusion step.

The % urinary recovery of PAH was calculated as described previously for inulin (chapter 2, p 93). AcPAH is expressed in terms of corrected PAH equivalents as described in section II, p 175, and % recovery was determined as described for PAH.

All clearances were corrected for a body surface area of 1.73 m^2

Statistical analysis

The statistical significance of differences between means was determined using 2 way analysis of variance. The null hypothesis was rejected if $p < 0.05$. Correlation coefficients were calculated by linear regression analysis.

RESULTS

Plasma concentrations

The plasma concentrations of PAH and AcPAH during each "step up" and "step down" infusion of PAH are given in Appendix IV, and the mean plasma concentrations are shown in Fig 3.3.2.

p-aminohippuric acid

During the "step up" infusion, the plasma concentrations appeared to reach steady state during the low, mid, and high periods. The respective mean mid-point plasma PAH concentrations were 4.4 $\pm$ 1, 8.5 $\pm$ 2 and 25.2 $\pm$ 5 mg/l.

With the "step down" study the mean midpoint plasma PAH concentrations were 22.8 $\pm$ 5, 9.6 $\pm$ 2 and 5.0 $\pm$ 1 mg/l for high, mid and low periods respectively. Steady state was not achieved during the mid and low periods.

Acetyl-p-aminohippuric acid

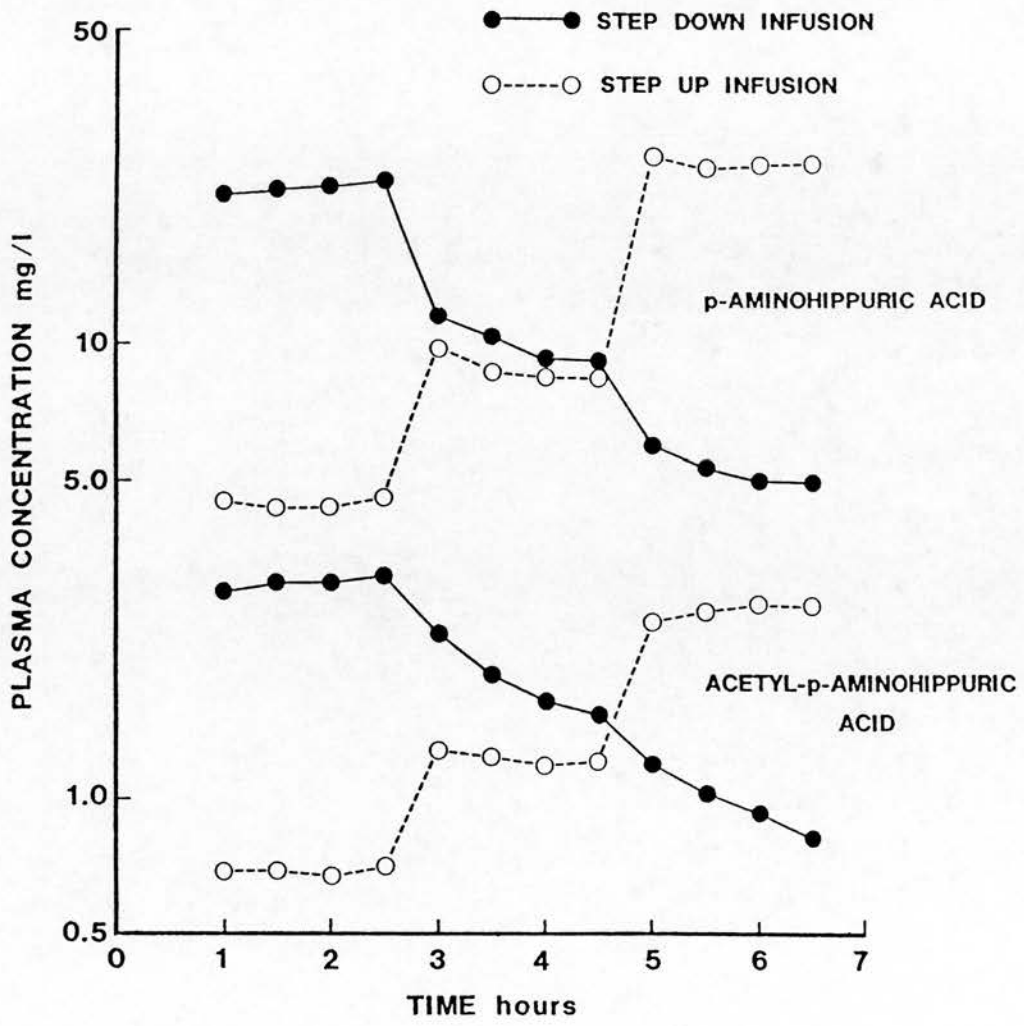
The mean AcPAH concentrations showed a similar trend to those of PAH, but at a much lower concentration. During the "step up" infusion, the plasma concentrations reached steady state during the low, mid, and high periods and the respective mean midpoint concentrations were 0.69 $\pm$ 0.2, 1.2 $\pm$ 0.4 and 2.6 $\pm$ 0.8 mg/l.

With the "step down" study, steady state was not attained, and the mean mid-point plasma AcPAH concentrations were 2.97 $\pm$ 0.7, 1.7 $\pm$ 0.4 and 0.9 $\pm$ 0.2 mg/l for high, mid and low periods respectively.

The ratio of the concentrations of the metabolite to the total plasma concentrations (PAH + AcPAH) decreased as the PAH plasma concentrations increased, and the mean values for the step up infusion were 11.5, 10.9, and 7.6 % and for the step down 12.9, 12.2, and 9.7 % for low, mid, and high periods respectively.

Fig 3.3.2

Mean plasma concentrations of PAH and AcPAH during step up and step down constant infusions in 8 healthy males.



Clearances

p-Aminohippuric acid

The individual renal clearances of PAH are given in Table 3.3.1 and the mean clearances in Fig 3.3.3. During the "step up" infusion, the mean PAH clearances increased progressively from 405 to 468 at low and mid and to 509 ml/min/1.73 m^2 at high plasma concentrations (Fig 3.3.3). This rise in clearance was significant over time and increasing plasma concentrations ($p< 0.01$), and the mean clearances for the low and mid periods were significantly lower than that at high plasma concentrations ($p<0.01$). Similarly, the mean clearance at the low period was significantly lower than that at the mid period ($p<0.05$).

Conversely, during the "step down" study the mean PAH clearances were 517, 506 and 379 ml/min/1.73 m^2 for high, mid and low plasma concentrations respectively (Fig 3.3.3). This fall in clearance was not statistically significant over time and declining plasma concentrations but, the mean clearance at the low plasma concentration was significantly less than at the high plasma concentration ($p<0.01$). Subject WW has an exceptionably high renal clearance for the middle period (1171 ml/min/1.73 m^2). With omission of the data from this subject, the mean renal clearances of PAH were 476, 412 and 344 ml/min/1.73 m^2 for high, mid and low periods respectively (Fig 3.3.3). This fall in clearance was significant over time and plasma concentration ($p< 0.01$).

A plot of the mean renal clearance of PAH against the mean mid point plasma concentrations for each period shows clearly that in the "step up" study, the clearance rose steeply as concentrations increased from the low to mid to the high period (Fig 3.3.4). The converse applied to the "step down" study but the renal clearance was similar during the mid to high periods (Fig 3.3.5).

TABLE 3.3.1

The renal clearance of PAH (ml/min/1.73 m^2) during step up and step down constant infusions of PAH in 8 healthy males.

	STEP UP INFUSION			STEP DOWN INFUSION		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
		PERIODS			PERIODS	
JN	392	386	395	393	403	375
GS	408	425	478	406	365	313
SB	394	437	495	441	380	363
AD	448	582	616	588	505	413
BB	437	436	449	526	491	326
SM	354	361	420	425	353	328
WW	474	681	689	807	1171	629
BW	330	436	529	551	384	288
MEAN	405	468	509	517	507	379
±SD	48	108	100	137	274	108
mean data after removal of WW				476	412	344
				78	61	43

Fig 3.3.3

Inverse relationship between the renal and total body clearances of PAH, and the renal clearances of AcPAH during step up and step down constant infusions of PAH in 8 healthy males.

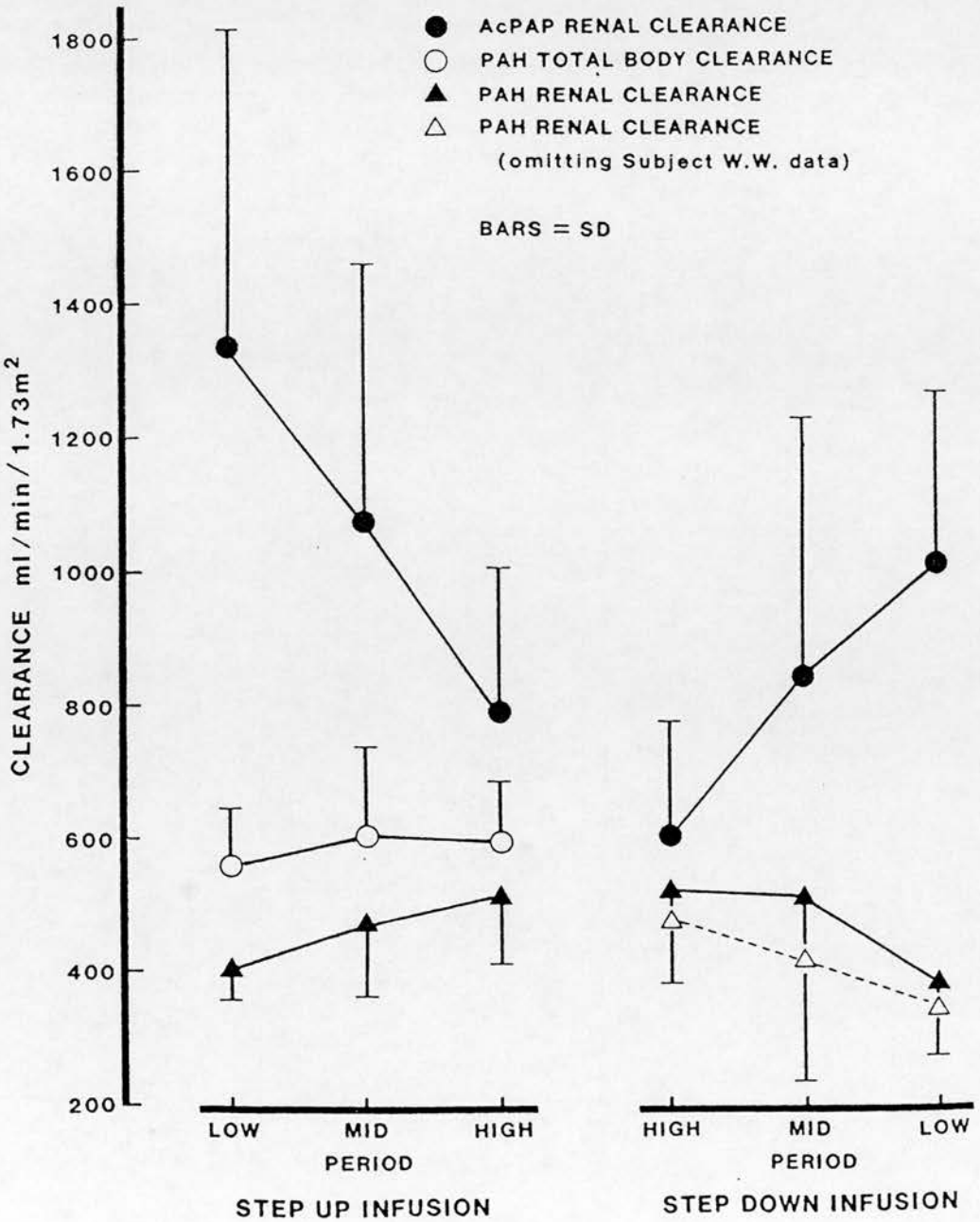


Fig 3.3.4

The relationship between the mean renal clearances of PAH (A) and AcPAH (B) and plasma concentrations of PAH during the step up constant infusion of PAH in 8 healthy males. Bars = ± SD.

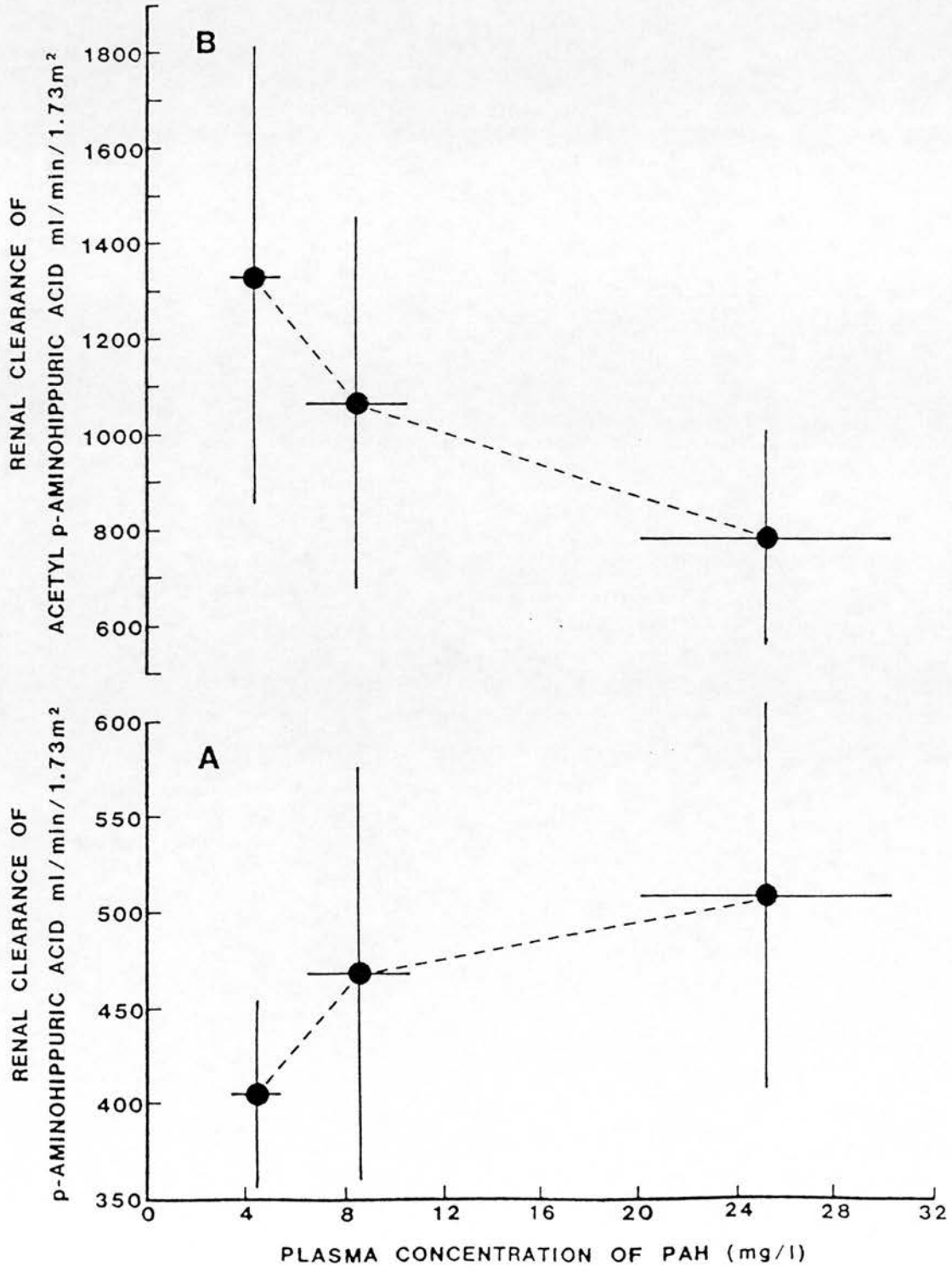
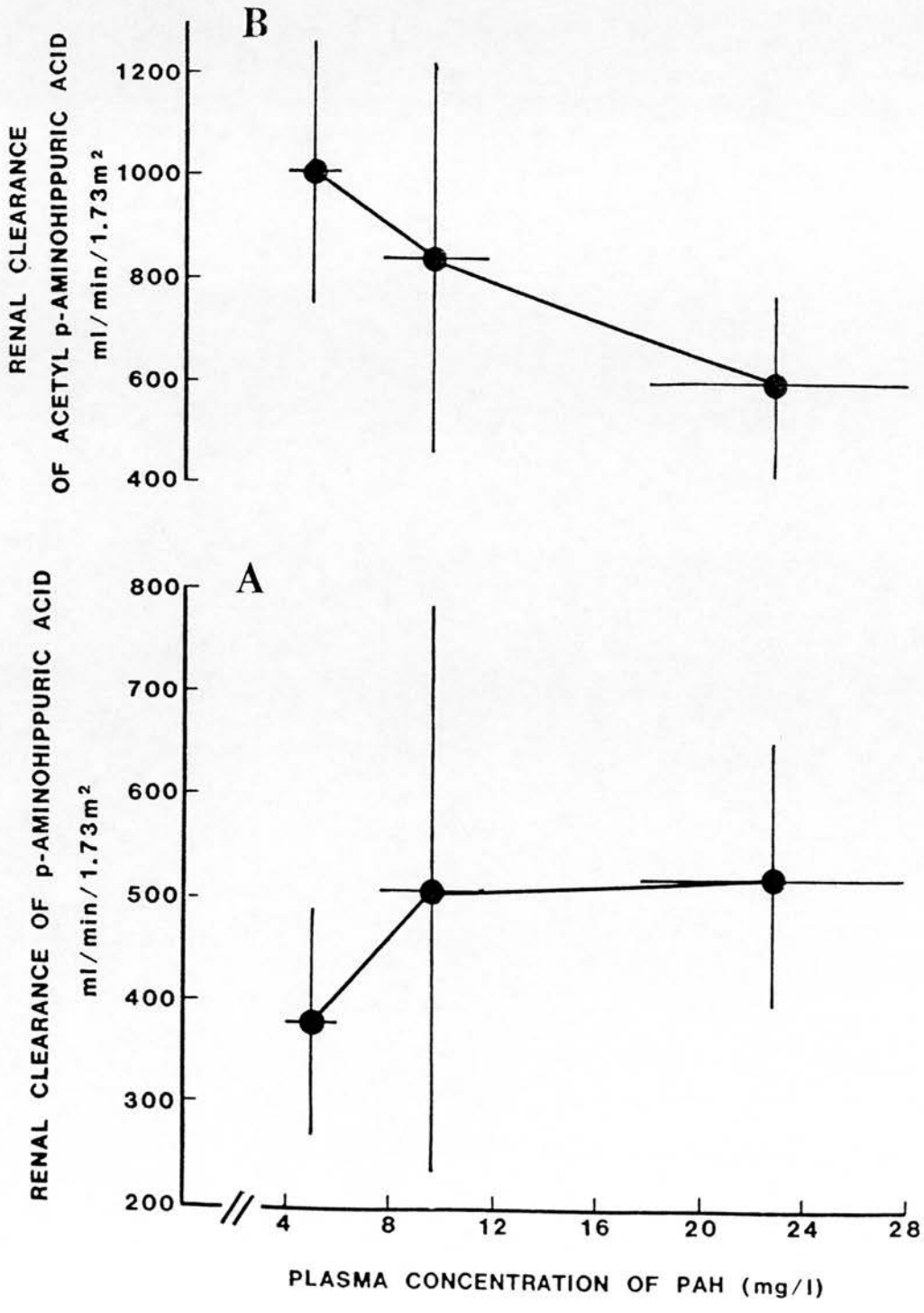


Fig 3.3.5

The relationship between the mean renal clearances of PAH (A) and AcPAH (B) and plasma concentrations of PAH during the step down constant infusion of PAH in 8 healthy males. Bars = ± SD.



Acetyl-p-aminohippuric acid

The individual renal clearances of AcPAH during the "step up" and "step down" infusions of PAH are given in Table 3.3.2 and the mean clearances are shown in Fig 3.3.3. During the "step up" infusion, the mean AcPAH clearance decreased progressively from 1334 to 1069 at low and mid to 782 ml/min/1.73 m^2 at high plasma PAH concentrations. This fall was significant over time and increasing PAH plasma concentrations ($p<0.01$), and the clearances at the low and mid periods were significantly higher than that at the high period ($p<0.01$). Similarly, the clearance at the low period was significantly higher than at the mid period ($p<0.01$).

Conversely during the "step down" study, the mean AcPAH clearances were 600, 845 and 1009 ml/min/1.73 m^2 for high, mid and low plasma concentrations respectively. This rise in clearance was also significant over time and declining PAH plasma concentrations and the clearances at low PAH plasma concentrations were significantly higher than at high plasma PAH concentrations ($p<0.01$). In subject WW, the AcPAH clearance was also very high during the mid period (1733 ml/min/1.73m^2) but when the data from this subject was omitted, the rise in clearance is still significant.

A plot of the mean renal clearances of AcPAH against the mean midpoint plasma PAH concentrations for each period shows that in the "step up", study the renal clearance of AcPAH fell steeply from low to mid plasma PAH concentrations, with a slower decline at the high concentrations (Fig 3.3.4). The opposite effect occurred in the "step down" study. The renal clearance of AcPAH increased as the plasma concentrations of PAH decreased from high to mid concentrations, and then rose steeply between the mid and low plasma PAH concentrations (Fig 3.3.5).

TABLE 3.3.2

The renal clearance of AcPAH (ml/min/1.73 m^2) during step up and step down constant infusions of PAH in 8 healthy males.

	STEP UP INFUSION			STEP DOWN INFUSION		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
		PERIODS			PERIODS	
JN	642	547	478	341	576	739
GS	1216	954	700	478	631	872
SB	1643	1126	826	516	672	1154
AD	1552	1375	992	768	1023	1244
BB	1156	849	632	685	775	779
SM	896	845	628	494	639	830
WW	2195	1834	1157	867	1733	1471
BW	1370	1019	845	652	708	987
MEAN	1334	1069	782	600	845	1009
$\pm$SD	478	391	219	173	384	258
mean data after removal of WW				562	718	943
				146	149	193

Total body clearance of PAH

The individual total body clearances of PAH are given in Table 3.3.3. The mean total body clearances in the "step up" study were 559, 601, and 590 ml/min/1.73 m^2 for low mid and high plasma concentrations respectively (Fig. 3.3.3). There were no significant differences with time or increasing plasma PAH concentrations.

During the "step down" study the total body clearance of PAH could not be measured as steady state conditions were not reached.

Comparison of clearances

The renal clearance of AcPAH was significantly greater than the corresponding renal clearance of PAH for all plasma PAH concentrations on both "step up" and "step down" studies ($p<0.01$).

A comparison of the mean renal clearances of PAH and AcPAH are shown below:-

	STEP UP			
	low	mid	high	
PAH CLEARANCE	405	468	509	ml/min/1.73 m^2
AcPAH CLEARANCE	1334	1069	782	ml/min/1.73 m^2

	STEP DOWN			
	high	mid	low	
PAH CLEARANCE	517	506	379	ml/min/1.73 m^2
AcPAH CLEARANCE	600	845	1009	ml/min/1.73 m^2

Significant correlations were found between the renal clearances of AcPAH and PAH for "step up" mid and high, ($r= 0.87$, $r=0.98$, $p<0.001$) and for "step down" low ($r= 0.79$, $p<0.05$), and mid and high ($r= 0.97$ and 0.92 respectively, $p<0.001$) periods respectively (Figs 3.3.6, and 3.3.7). There was no significant correlation between clearances for the low period in the "step up" study.

During the "step up" study, the total body clearance

TABLE 3.3.3

The total body clearance of PAH (ml/min/1.73 m^2) during step up constant infusion of PAH in 8 healthy males.

	STEP UP INFUSION		
SUBJECT	LOW	MID	HIGH
	PERIODS		
JN	591	544	526
GS	571	528	577
SB	619	615	627
AD	665	710	670
BB	513	526	507
SM	472	452	500
WW	631	883	769
BW	411	555	551
MEAN	559	602	591
±SD	87	136	93

Fig 3.3.6

The relationship between the renal clearances of AcPAH and PAH during the step up constant infusion of PAH in 8 healthy males.

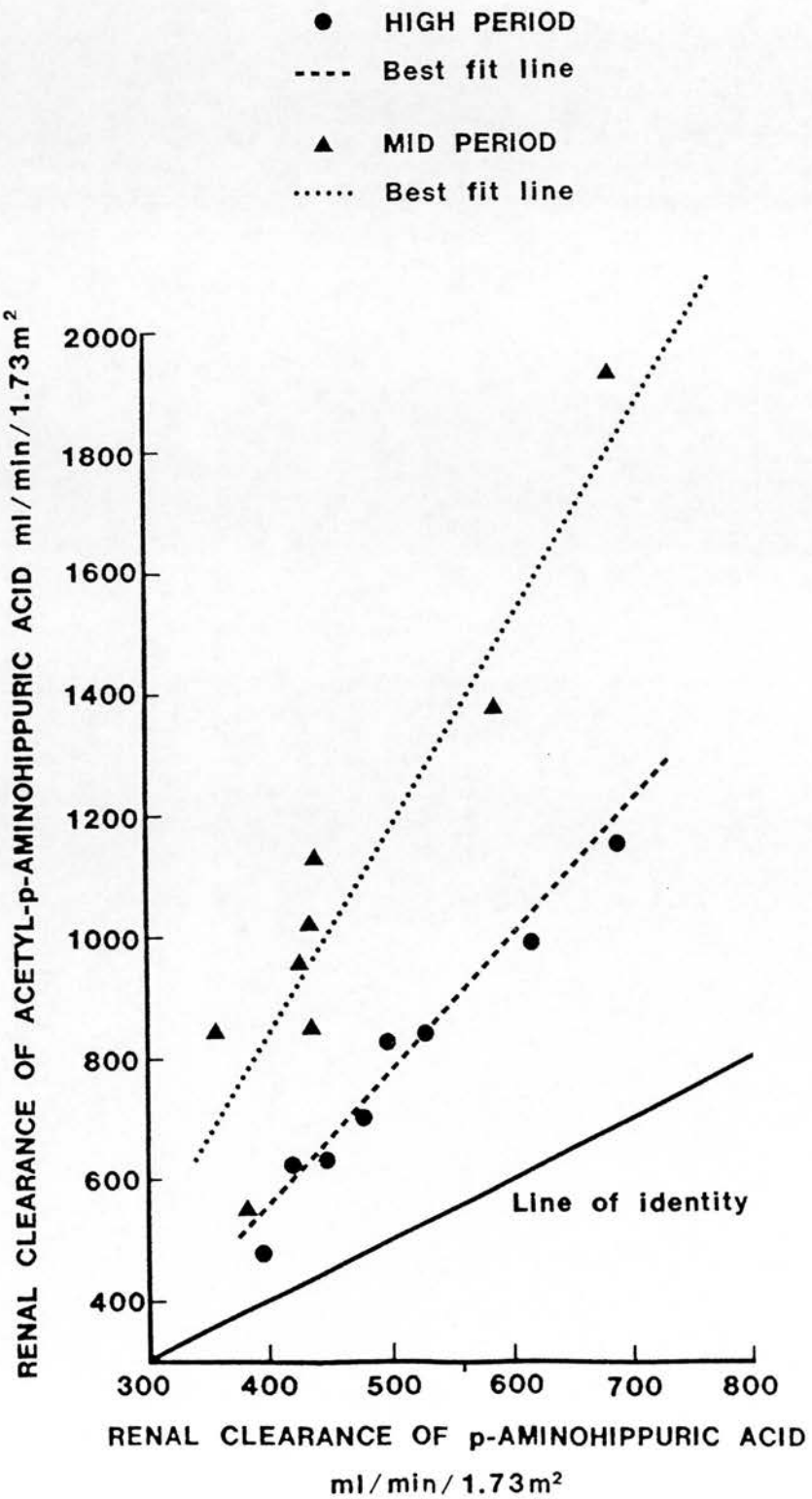
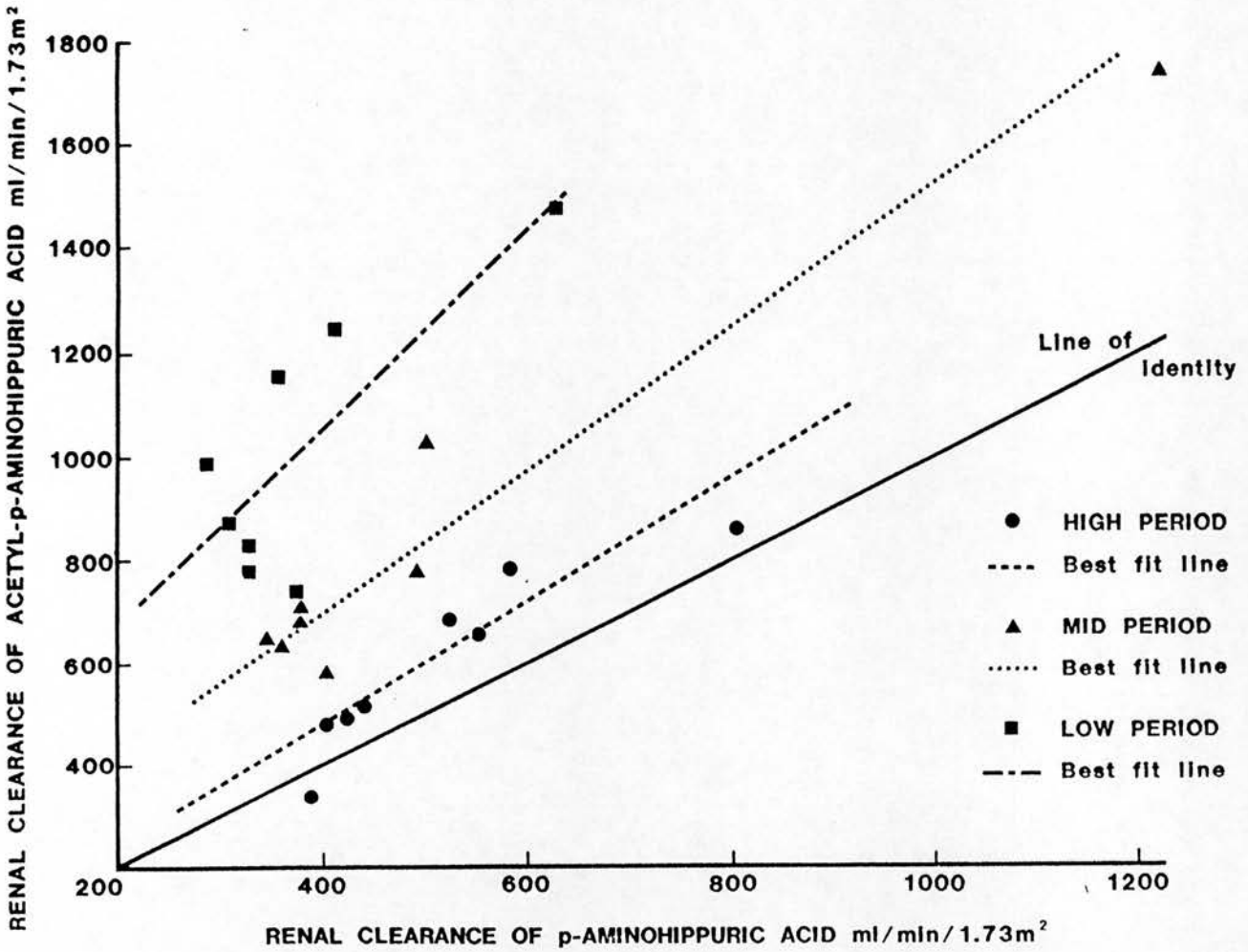


Fig 3.3.7
Relationship between the renal clearances of AcPAH and PAH during the step down constant infusion of PAH in 8 healthy males.



of PAH was significantly higher than the renal clearance (559 v 405; 602 v 468 and 591 v 509 ml/min/1.73 m^2) for the low, mid and high plasma PAH concentrations respectively, ($p<0.01$) but it was significantly less than the AcPAH renal clearance (559 v 1334; 602 v 1069 and 591 v 782 ml/min/1.73 m^2) for low, mid and high plasma concentrations respectively, ($p<0.01$).

Urine flow rate

The individual urine flow rates for each period during the two study days were similar (Table 3.3.4). There were no significant correlations between the PAH or AcPAH renal clearances and the corresponding urine flow rates.

Urinary recovery

The urinary recoveries of PAH and AcPAH for both studies are given in Table 3.3.5.

p-aminohippuric acid

For the "step up" study, the mean percentage recovery of PAH relative to the amount infused increased from 72 $\pm$ 6 to 78 $\pm$ 5 and 85 $\pm$ 7 % for the low, mid and high periods respectively (Fig 3.3.8). For the "step down" study, the recoveries of PAH were 82 $\pm$ 8, 95 $\pm$ 18 and 79 $\pm$ 9 % for high, mid and low periods respectively (Fig 3.3.8). The mean amounts of PAH excreted as percentages of the total (PAH + AcPAH) were 72 $\pm$ 5, 80 $\pm$ 3 and 89 $\pm$ 2 % for the "step up" and 71 $\pm$ 4, 80 $\pm$ 2 and 89 $\pm$ 1 % for the "step down" studies in the low, mid and high periods respectively. Thus during the corresponding time periods, the total recoveries were virtually identical in both studies.

Acetyl-p-aminohippuric acid

For the "step up" study, the mean percentage recoveries of AcPAH relative to the amount infused, decreased progressively from the low to mid to high

TABLE 3.3.4

Urine flow rate (ml/min) during step up and step down constant infusions of PAH in 8 healthy males.

	STEP UP INFUSION			STEP DOWN INFUSION		
SUBJECT	LOW	MID	HIGH	HIGH	MID	LOW
		PERIODS			PERIODS	
JN	7.2	7.9	8.8	9.6	5.9	10.1
GS	8.1	8.7	8.5	8.1	9.5	7.5
SB	9.6	4.4	9.6	4.1	4.8	7.6
AD	7.6	9.3	6.7	8.3	8.5	8.6
BB	11.0	9.5	10.0	9.4	7.0	8.5
WW	11.7	8.9	8.0	12.3	12.3	12.7
SM	7.8	7.0	6.8	8.6	6.9	8.4
BW	6.4	5.0	8.9	7.5	6.1	6.1
MEAN	8.7	7.6	8.4	8.5	7.6	8.7
$\pm$SD	1.9	2.0	1.2	2.3	2.4	2.0

TABLE 3.3.5.

The percentage urinary recoveries of PAH and AcPAH relative to dose administered in 8 healthy males during step up (A) and step down (B) constant infusions of PAH.

A)

STEP UP

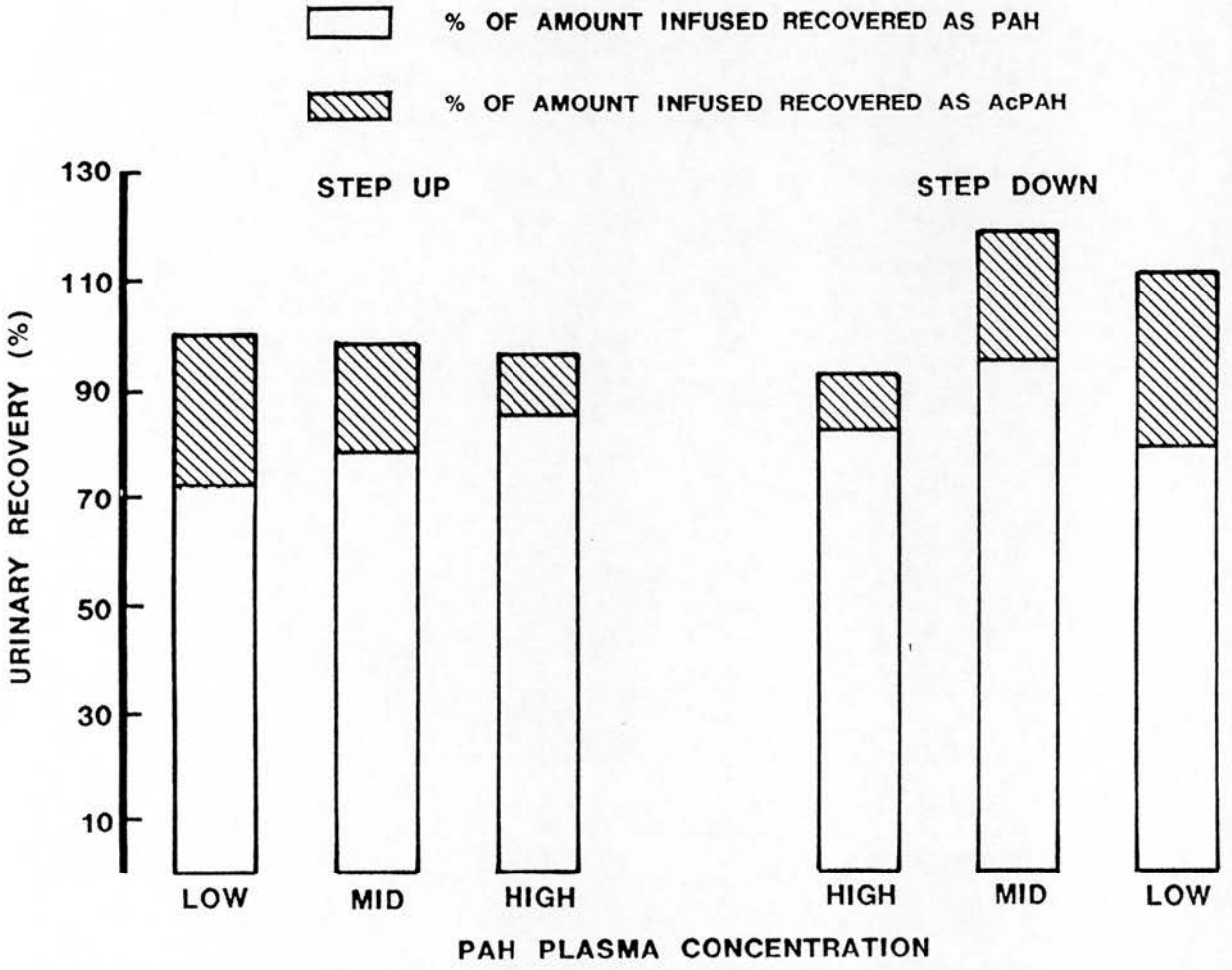
SUBJECT	LOW		MID		HIGH	
	PAH	AcPAH	PAH	AcPAH	PAH	AcPAH
JN	65.2	26.6	70.4	18.4	74.8	10.3
GS	71.8	24.7	80.3	15.7	83.0	7.8
SB	63.0	39.8	69.8	27.7	77.5	13.3
AD	73.0	30.0	82.0	22.7	95.0	12.2
BB	83.0	27.3	84.3	20.8	85.1	11.3
SM	75.0	25.2	79.3	19.9	85.0	10.9
WW	71.6	29.1	82.9	19.0	85.6	9.1
BW	73.9	24.1	78.6	16.7	94.4	10.5
MEAN	72.1	28.4	78.5	20.1	85.1	10.7
±SD	6.1	5.1	5.5	3.8	7.1	1.7

B)

STEP DOWN

SUBJECT	LOW		MID		HIGH	
	PAH	AcPAH	PAH	AcPAH	PAH	AcPAH
JN	67.4	8.8	83.7	22.5	78.1	29.7
GS	73.2	9.0	79.4	19.8	71.4	28.7
SB	84.0	11.9	98.2	25.1	94.9	48.1
AD	88.0	11.8	87.0	23.9	74.0	32.7
BB	90.0	11.5	103.4	22.7	74.1	26.8
SM	81.4	8.8	88.4	19.5	87.5	30.0
WW	89.7	8.4	134.9	28.7	84.5	24.8
BW	83.6	9.7	82.3	20.6	66.4	34.8
MEAN	82.2	10.0	94.7	22.9	78.9	32.0
±SD	8.1	1.5	18.2	3.1	9.4	7.2

Fig 3.3.8
Mean percentage urinary recovery of PAH and AcPAH in relation to the amount infused during step up and step down constant infusion of PAH in 8 healthy males.



(28 ± 5, 20 ± 4 and 11 ± 2 % respectively; Fig 3.3.8).

For the "step down" study, the mean recoveries of AcPAH were 10 ± 2, 23 ± 3 and 32 ± 7 % for the high, mid and low periods respectively (Fig 3.3.8). The recoveries of AcPAH as a percentage of the total amount excreted (PAH + AcPAH), were 28 ± 5, 20 ± 3 and 11 ± 2 % for the "step up" and 29 ± 4, 20 ± 2 and 11 ± 1 % for the "step down" studies during the low, mid and high periods respectively.

The total recovery of PAH and AcPAH in relation to the amount infused during each collection period were 100 ± 5, 99 ± 5 and 96 ± 7 % for the low, mid and high periods during the "step up" study and the corresponding values for the step down study were 92 ± 9, 118 ± 21 and 111 ± 14 % during the high, mid and low periods (Fig 3.3.8).

DISCUSSION

These results show clearly that the renal clearance of PAH changes significantly depending on its plasma concentration. As the plasma concentrations of PAH increased from 4.4 to 25 mg/l, the mean renal clearance of PAH rose by 20 % and this was significantly related to time and increasing plasma concentrations. At the same time, the renal clearance of AcPAH decreased by 41%. Conversely, as the plasma PAH concentrations decreased during the step down infusion, the clearance of PAH decreased by 27% and the renal clearance of AcPAH increased by 41%. As expected there were significant correlations between the renal clearances of PAH and AcPAH, except during the low period of the step up infusion. The total body clearance of PAH during the "step up" infusion remained constant over the three collection periods and was significantly greater than the renal clearance. The difference was greater at low plasma PAH concentrations (26 %), than at high plasma PAH concentrations (13 %).

The changes in renal clearances were clearly related to plasma concentration of PAH and time can be excluded as a significant factor. As steady state plasma concentrations of PAH were achieved in the "step up" infusion, the changes in PAH clearance cannot be due to arterio-venous differences, as these should be minimal under steady state conditions (Brun et al, 1949). This may not hold for the "step down" study, as steady state was not reached. A greater total body than renal clearance of PAH during constant infusion (20-30 %) has been reported previously (Statius Van Eps et al, 1967; Cole et al, 1972). This difference was similar to that found in the present study at low plasma PAH concentrations, but greater than that found at high plasma concentrations. However, the plasma concentrations in these previous reports were similar to the high plasma

PAH concentrations achieved in the present study, and the differences can probably be explained by their use of subjects with impaired renal function. Other investigators have not observed differences between the total body and renal clearances of PAH during constant infusion (Berger et al, 1948; Schnurr et al, 1980), but this can be accounted for by the use of methods involving hydrolysis. This would convert AcPAH back to PAH.

The consistently higher total body clearance of PAH indicates significant extrarenal clearance, which is greater at low than at high plasma PAH concentrations. This can be accounted for entirely by the acetylation of PAH, as the total recovery of PAH and AcPAH was 100 % of the amount infused during the step up study. These findings confirm the findings in the single injection study that both PAH and AcPAH, were completely excreted by the kidney. Under the conditions of constant infusion, AcPAH was the major or even sole metabolite of PAH. During the "step down" infusion, the total recovery exceeded the amount infused after the high period and this reflects redistribution with failure to reach steady state conditions.

The reciprocal changes in the clearances of PAH and AcPAH were directly related to the concentration dependant clearance of PAH. No substance can have a renal clearance greater than the renal plasma flow, but the clearance can be lower if the extraction ratio is reduced (Smith, 1951). It is possible that at low plasma concentrations, PAH and AcPAH do not reflect the clearance from whole blood due to uptake into erythrocytes or increased plasma protein binding.

Uptake into erythrocytes

If concentrations of a substance are only measured in plasma, irreversible uptake into erythrocytes could cause an artificially low clearance. In this case the

uptake of PAH and AcPAH into erythrocytes seems unlikely as this does not occur, to a significant extent, "in vitro" or "in vivo" (Smith et al, 1945).

Plasma protein binding

Increased plasma protein binding, at low plasma concentrations has previously been put forward as an explanation for the depression in diodrast clearance at low plasma concentrations (Block & Burrows, 1960). There is evidence that the fraction of PAH bound to plasma proteins increases at low concentrations (Taggart, 1951). However, PAH is reported not to be strongly bound to plasma proteins and to have a great affinity for the tubular transport system (Moller & Sheikh, 1983). The plasma protein binding of AcPAH does not seem to have been investigated, but with decreasing plasma concentrations, the extraction ratio is apparently constant (Newman et al, 1949). Therefore, a change in plasma protein binding seems an unlikely explanation for the changes seen in the renal clearances of PAH and AcPAH.

The excretion of PAH is usually described by two intrarenal processes, filtration and secretion. However, there are 2 other major determinants of excretion that can effect the clearance of a compound. These are reabsorption, and intrarenal metabolism (Weiner, 1985). If either of these renal mechanisms are effecting the excretion of PAH, the renal clearance of PAH will be altered as it is the resultant of all intrarenal processes. An effect on filtration seems unlikely as the creatinine clearance remained unchanged, and would therefore not explain the plasma concentration dependant clearance of PAH.

As PAH and AcPAH are excreted via the same organic anion transport system, it is possible that PAH excretion is reduced due to competition for this system. This seems unlikely considering the concentration differences between them but it cannot be ruled out. The

possible role that tubular reabsorption and intrarenal metabolism may have on the renal clearance of PAH are discussed below:-

Tubular reabsorption

Tubular reabsorption of PAH has been reported to occur in dogs and rats (Cho & Cafruny, 1970; Moller & Sheikh, 1983). In man a rapid decline in renal clearance of two cephalosporin antibiotics, (cephapirin and cephaloridine) similar to that seen with PAH following a single injection, has been shown to be due to saturable tubular reabsorption (Arvidsson, 1982). Saturable tubular reabsorption of PAH would be consistent with reduced renal clearance, and proportionately reduced urinary excretion of PAH at low plasma concentrations. However, this will not explain the inverse relationship between the renal clearances of AcPAH and PAH as plasma concentrations decline. Bidirectional transport with saturable tubular reabsorption of PAH cannot be ruled out but this mechanism alone cannot account for the findings.

Renal metabolism of PAH

Acetylation of PAH in the kidney would be consistent with the lack of change in the total body clearance of PAH during the "step up" infusion. This would also explain why the AcPAH, clearance after administration of PAH, was so much greater than reported in other studies when given alone (Smith et al, 1945; Newman et al, 1949). The renal metabolism of PAH will increase the amount of AcPAH and decrease the amount of PAH present in the urine, irrespective of the plasma concentration. In such circumstances, the apparent renal clearance of AcPAH will be spuriously increased while the renal clearance of PAH will be reduced. Such an effect would also be consistent with the changing renal clearance of PAH, whilst the total body clearance of PAH remained

constant. The difference between total body and renal clearance of PAH become less as the plasma concentrations of PAH increased, and the apparent renal clearance of AcPAH increased dramatically at low plasma concentrations of PAH. Taken together, these findings suggest saturable renal acetylation of PAH.

Renal metabolism should be suspected if the apparent renal clearance of a metabolite, following administration of the parent drug, is much greater than that when the metabolite is given alone (Tucker, 1981). AcPAH is only removed by renal excretion, and its clearance cannot be greater than the renal plasma flow. In the present study, the renal clearance of AcPAH at low plasma PAH concentrations was greater than the expected physiological renal plasma flow. In other studies, the renal clearances of AcPAH given alone and PAH are reported to be similar (Smith et al, 1945; Newman et al, 1949; Statius Van Eps et al, 1967). The reliability of these findings must, however, be open to question because of the indirect methods used for analysis. Stolk et al, (1985), investigated a new HPLC technique for measuring AcPAH, and during a constant infusion of AcPAH (10 mg/min) in volunteers, described steady state plasma concentrations, of 18, 21, and 23 mg/l, but unfortunately no clearances were reported. On the assumption that AcPAH has a maximal clearance and high extraction ratio, it can be calculated from the cited infusion rate and steady state concentrations that the total body clearances of AcPAH were 555, 476 and 435 ml/min respectively. As no conditions were given, and the clearance of PAH in their subjects is unknown, these results are of limited value. However, AcPAH is totally excreted by the kidney, as shown in the present study and it is not deacetylated in man (Newman et al, 1949). The clearance values for AcPAH calculated from the data of Stolk et al, (1985), are well below those observed in

the present study. This again suggests that the high apparent AcPAH clearances found in the present study reflect renal acetylation of PAH and are therefore, not a true measure of the renal blood flow.

Acetylation of PAH has been demonstrated in animals (Setchell & Blanch, 1961), and also "in vitro" in human kidney slices (Frindt & Vial, 1968). In this latter study, the kidney had a greater capacity for acetylation of PAH in man than the liver, and saturation of acetylation was demonstrated. Saturation of kidney metabolism also occurs in mouse kidney slices (Carpenter & Mudge, 1980). Smith (1951) cited studies by Hamburger and Ryckewaert in which the PAH clearances at plasma concentrations below 10 mg/l were very low, after single injection and during constant infusion. This was attributed to tubular conjugation, high plasma protein binding or tubular "inertia". Newman et al, (1949), also thought that PAH was metabolised in the kidney in man because its renal clearance fell in man, but not in dogs (who cannot acetylate) following a single injection. The proportion of conjugate in urine increased as the plasma concentrations of PAH decreased, as observed in the present study. Grindt et al, (1974), also suggested that PAH was metabolised in the kidney. On the other hand, Smith (1951) stated that although conjugation in the kidney could not be excluded, significant renal conjugation at low plasma concentrations was unlikely, and that extrarenal metabolism would have no effect on the renal clearance of PAH. Pearson (1979) reports that the presence of AcPAH does not interfere with the estimation of ERPF.

The weight of evidence from the present studies, supported in part by previous reports, indicate either renal metabolism of PAH or interference with its excretion as the cause of the significant decrease in the renal clearance of PAH, at low plasma concent-

rations. Either way, the reduced clearance of PAH at low plasma concentrations seems to be related to its metabolism to AcPAH.

Berger et al, (1948), proposed a constant infusion method without urine collection, reporting similar total body and renal clearances of PAH. They took into consideration the metabolism of PAH and converted AcPAH back to PAH by hydrolysis. This method has potential problems due to the formation of degradation products (Brown et al, 1976). However, the total body clearance i.e. PAH + AcPAH (as PAH equivalents) can be estimated by summing the PAH and AcPAH concentrations (as PAH equivalents) in plasma and urine to give the total body clearance of PAH + AcPAH and renal clearance of PAH + AcPAH, without the errors involved with hydrolysis. In this way, the total body and renal clearances of PAH + AcPAH in the present "step up" infusion study were, similar (Table 3.3.6).

TABLE 3.3.6

Mean (± SD) clearances of PAH and total PAH + AcPAH during the "step up" infusion study

PERIOD	Rclt	TBCt	Rclt/TBCt	Rclp	Rclt/Rclp
	PAH + AcPAH			PAH	
LOW	497 (73)	494 (75)	1.00 (0.07)	405 (48)	1.23 (0.09)
MID	525 (129)	540 (127)	0.97 (0.05)	468 (108)	1.12 (0.03)
HIGH	531 (109)	546 (92)	0.97 (0.08)	509 (93)	1.04 (0.02)

t = PAH + AcPAH p= PAH

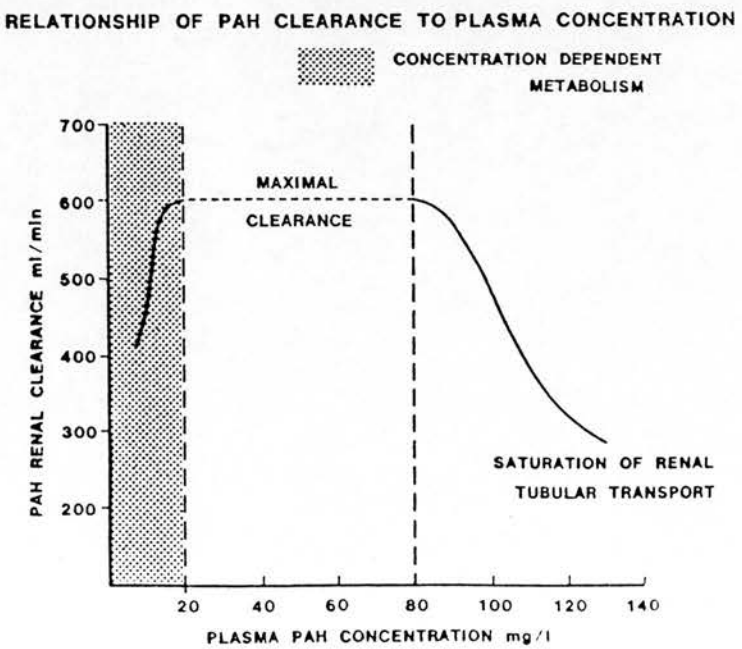
Rcl = renal clearance TBC = total body clearance

If the renal clearance of total PAH + AcPAH is taken as the best measure of the renal plasma flow it should

be equal to the renal clearance of PAH, in the absence of renal metabolism. This is not the case, as the renal clearance of PAH is consistently less than the clearance of PAH + AcPAH, at low PAH plasma concentrations (Table 3.3.6). This discrepancy becomes less as the plasma concentrations of PAH rise. Presumably at some higher plasma concentration, the renal clearance of PAH and PAH + AcPAH will become almost equal. At this point, the renal acetylation of PAH would be saturated. PAH is probably also metabolised extrarenally, presumably in the liver.

The clearance of PAH is dependent upon its plasma concentration and therefore, its use for measuring renal plasma flow may only be valid over a narrow range of plasma concentrations, where the clearance is maximal. This range is above the point above where renal PAH metabolism becomes saturated, and below the level of saturation of its renal tubular transport. Thus the classical representation of the renal clearance of PAH in relation to plasma concentrations (shown on page 35), should be redefined as follows:

Fig 3.3.9



From the present data, it is not possible to define the plasma concentrations at which there is complete saturation of PAH metabolism (and possibly tubular reabsorption), but with measurement of the renal clearance of PAH by the constant infusion technique, plasma concentrations should be kept above 20 mg/l.

The fall in PAH clearance following single injection was more dramatic than observed in the present "step up" and "step down" constant infusion, and this is probably due to the much lower plasma concentrations in the 1-2 h period. In addition, the fall in clearance may have been exacerbated by other factors such as arterio-venous differences or delay time. The results of the present study suggest that at PAH plasma concentrations above 20 mg/l, the effects of renal metabolism are small. The plasma concentrations achieved during the constant infusion (section II, p 178) were above 20 mg/l and the clearances were similar to the 0-1 hour renal clearances of PAH following single injection. Therefore, the 0-1 hour renal clearance of PAH is probably a better estimate of the effective renal plasma flow than the total body clearance. The major disadvantage of this method is that accurate urine collections are still required. This may be overcome by infusing to steady state plasma concentrations and measuring the total body clearance only. An appropriate correction factor could be applied to correct for the fraction converted to AcPAH extrarenally, providing the plasma concentrations of PAH were above the point of significant renal metabolism. Urine would still have to be collected but all that is required is the fraction of the dose converted extrarenally.

It is clearly time to reevaluate the role of PAH as the standard for measurement of the renal plasma flow. Its clearance is dependant on plasma concentration, and it is probably metabolised as it passes through the

kidney. In the present study there was no evidence of genetically controlled acetylation of PAH as reported for other arylamines (Weber & Hein, 1985), but the number of subjects studied was small. If there is genetic polymorphism of PAH acetylation, estimates of renal plasma flow may depend on whether the subject is a fast or slow acetylator. If AcPAH has a high maximal extraction ratio and its total body and renal clearances are similar (Smith et al, 1945; Newman et al, 1949; Statius Van Eps, 1967), it should be a much better test compound than PAH for measuring renal plasma flow. It may not have been introduced previously because of the lack of a specific assay.

SUMMARY

The renal clearance of PAH falls as the plasma concentration declines, whilst the proportion of PAH acetylated increases. The opposite is found to occur as the plasma concentrations of PAH increase. The renal clearance of AcPAH is inversely related to that of PAH. The total body clearance of PAH and the total PAH + AcPAH renal clearance remained relatively constant on increasing the plasma concentration of PAH. Decreased extraction due to plasma protein binding and uptake into erythrocytes, are thought to be unlikely explanations for the fall in the renal clearance of PAH. However, saturable tubular reabsorption of PAH could contribute to the decline in PAH renal clearance, but does not explain all the results. The most plausible explanation behind the plasma concentration dependant clearance of PAH is saturable concentration dependant renal acetylation.

The renal clearance of PAH is plasma concentration dependant below 20 mg/l. Therefore, any method used to measure the renal clearance of PAH below this level, will be inaccurate. Above these levels urine must be collected, due to significant extrarenal clearance.

AcPAH should be investigated as a possible replacement of PAH to measure renal blood flow.

SECTION IV

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The disposition and kinetics of p-aminohippuric acid (PAH) and its metabolite acetyl p-aminohippuric acid (AcPAH) were investigated following single intravenous administration and during constant infusion of PAH. PAH and AcPAH were estimated using specific high performance liquid chromatographic assays.
The results obtained and conclusions drawn can be summarised as follows:-

1) Following single intravenous bolus administration of PAH (10 mg /Kg) in 26 healthy males, the total body and renal clearances of PAH fell significantly and dramatically (a mean fall of 54 % in the case of the renal clearance) in the second hour compared to the first. Eighty two percent of the administered dose of PAH was recovered in the urine as PAH, whilst the remainder was recovered as AcPAH.

2) During "step up" and "step down" constant infusions, the PAH renal clearance decreased significantly as steady state plasma concentrations decreased, well below the level of saturation of the renal transport system. However, the total body clearance of PAH during the "step up" study did not alter, suggesting an intrarenal mechanism behind the fall in the PAH renal clearance.

3) The total body clearance of PAH was significantly greater than its renal clearance following a single injection (14 %, 0-1 h), and during constant infusion. During the "step up" constant infusion, the difference between total body and renal clearance of PAH was greater at low (26 %) than at high (13 %) PAH plasma concentrations. This difference between the total body

and renal clearances of PAH is due to its metabolism to AcPAH.

4) AcPAH was present in plasma and urine following both single intravenous injection and constant infusion of PAH. In all studies, the renal clearance of AcPAH was significantly greater than the corresponding renal clearance of PAH. During the "step up" and "step down" constant infusion, the changes in AcPAH clearance were inversely related to the changes in PAH clearance.

6) The renal clearance of PAH + AcPAH (as PAH equivalents) remained relatively constant during the "step up" study, but was significantly greater than the renal clearance of PAH at low plasma concentrations. The differences between the two clearances decreased as the PAH plasma concentration increased. This suggests that the renal clearance of PAH is effected by its metabolism, which is greater at low plasma concentrations of PAH.

7) The renal clearance of PAH is clearly dependent on the plasma concentration, and decreases progressively as concentrations fall below 20 mg/l. This is unlikely to be due to decreased renal extraction because of increased plasma protein binding or uptake into erythrocytes at low concentrations.

8) The effects of arterial-venous differences and the delay time can also be ruled out as causes, because the renal clearance changes significantly in the "step up" infusion where these factors are minimal.

9) The concentration dependant decrease in the renal clearance of PAH could be explained by saturable tubular reabsorption. However, the weight of evidence suggests

that PAH is metabolised as it passes through the kidney, and such a mechanism is consistent with the results of these studies.

10) The renal clearance of PAH was similar following a single injection as during constant infusion, in the same 10 healthy males.

11) The single injection method has limited application for measurement of the renal clearance of PAH because it undergoes significant metabolism. The extent to which PAH is metabolised in the kidney could not be determined in the present study, but this process probably becomes saturated at high plasma concentrations.

12) During the first hour following a single intravenous injection of PAH, the renal clearance of PAH is similar to that during constant infusion (465 v 444 ml/min/1.73 m^2 respectively), and the method can be used as long as urine is collected.

13) The renal clearance of PAH decreases progressively as the plasma concentrations fall below 20 mg/l and this effect can be largely explained by concentration dependant renal acetylation. Therefore, any method used to measure the renal clearance of PAH will be inaccurate at low plasma concentrations, and above these levels there will be significant extrarenal clearance.

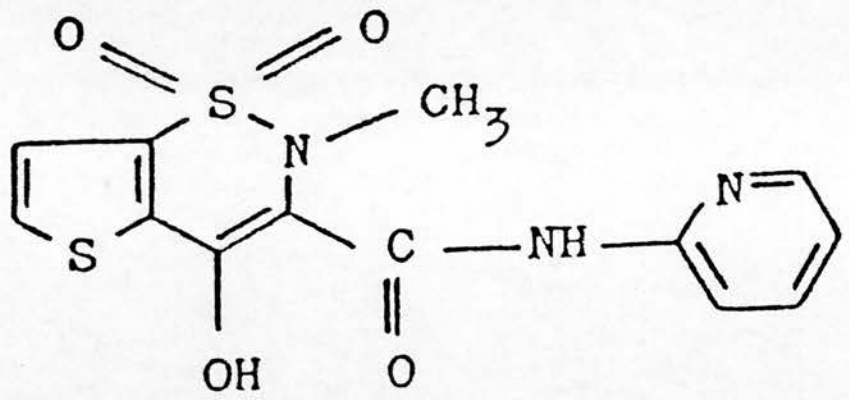
CHAPTER FOUR

THE EFFECTS OF TENOXICAM ON RENAL FUNCTION

INTRODUCTION

Tenoxicam is a new non-steroidal anti-inflammatory drug (NSAID). It is a thienothiazine derivative, and belongs to the oxicam class (Fig. 4.1).

Fig 4.1



Structure of Tenoxicam MW 337.4

Chemical name: 4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno-[2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide.

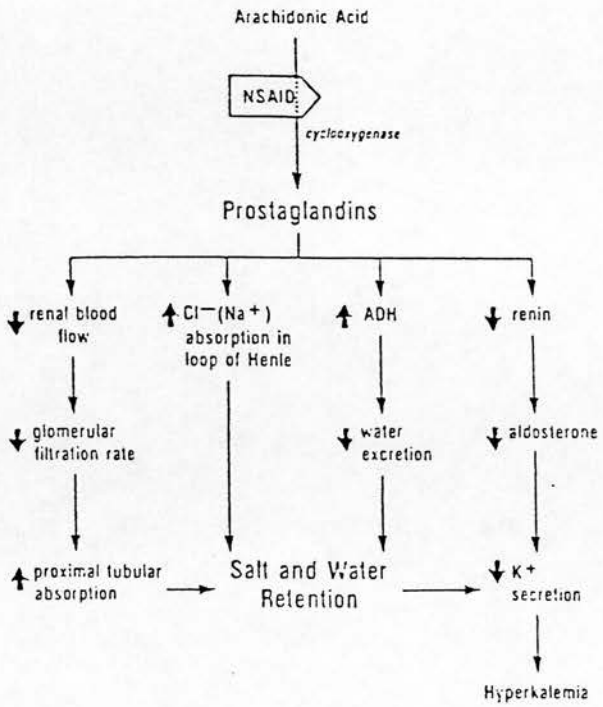
Its anti-inflammatory and analgesic properties have been demonstrated in animal models, and in patients with rheumatoid arthritis and osteo-arthritis. Tenoxicam has a long half life of about 72 hours, allowing once daily administration and steady state plasma concentrations are achieved by 12 to 15 days. The precise mode of action of tenoxicam is unknown, but it inhibits prostaglandin synthesis and reduces leucocyte accumulation and phagocytosis (Gonzalez & Todd, 1987).

Prostaglandins are cyclic derivatives of the fatty acid arachidonic acid and they are synthesised locally, exerting their action near this site. Arachidonic acid is oxygenated by cyclo-oxygenase enzymes present in the endoplasmic reticulum to form the enderoperoxides PGG2 and PGH2, and these are then converted enzymatically to the biologically active prostaglandins. Arachidonic acid can also be converted to leukotrienes by lipooxygenase

(Dunn, 1984).

The group of non-steroidal anti-inflammatory drugs includes indomethacin, ibuprofen, naproxen and piroxicam. These have all been linked with pathological and physiological changes in renal function including reduction in renal blood flow, chronic renal disease, papillary necrosis and also unwanted sodium, potassium and fluid retention. These effects are thought to be mediated by inhibition of cyclo-oxygenase, and reduced synthesis of renal prostaglandins (Fig.4.2) (Carmichael & Shankel, 1985; Adams et al, 1986; Clive & Stoff, 1984).

Fig 4.2



Changes in renal function following NSAID administration
ADH = antidiuretic hormone. (Carmichael & Shankel, 1985)

Tenoxicam has been reported to be as effective and as well tolerated as indomethacin, ibuprofen and naproxen in clinical studies, and in animal models of inflammation, tenoxicam was as potent as piroxicam and indomethacin (Gonzalez & Todd, 1987). However, it has

been postulated that non-steroidal anti-inflammatory drugs which have long elimination half lives, have a greater potential for renal side effects than those with short half lives (Adams et al, 1986).

The aim of this study, was to determine the effect of tenoxicam on renal function in healthy volunteers as assessed by measurement of the glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and urinary prostaglandins and electrolyte excretion. Urinary N-acetyl-glucosamidinase and B_2 microglobulin were also measured as indicators of acute renal tubular damage (Prescott, 1982a). Studies were carried out before, and during administration of tenoxicam over a period of ten days.

METHODS

Volunteers

16 healthy male volunteers aged between 22 and 42 years (mean age 27 $\pm$ 5 yrs) weighing 57 - 88 Kg (mean 70 $\pm$ 8 Kg), were recruited. A complete medical examination was performed, and the results of routine blood biochemistry and haematology screening were within normal limits. The study was approved by the local ethical committee, and written consent was obtained from each volunteer. All subjects were instructed to avoid alcohol and excessive salt intake during the study, and to refrain from sexual activity 36 hours before each study day, to avoid urinary contamination with prostaglandins.

Study design

The study was a randomised, double blind, placebo-controlled parallel group study. Each subject received two tablets of tenoxicam (40 mg) or placebo on days one and two, followed by one tablet of tenoxicam (20 mg) or placebo for the next eight days. There were eight subjects in each group. Detailed tests of renal function

were performed before dosing, and on the third and tenth day of drug administration (Fig 4.3).

On each study day, the volunteers had a light breakfast without caffeine containing drinks before attending hospital. On arrival, they emptied the bladder, were weighed, and blood was taken for biochemistry and estimation of plasma tenoxicam concentrations. 500 ml of water was then drunk over 5 minutes, and on days 3 and 10 tenoxicam or placebo was administered, at this time. After the initial water loading, the volunteers drank 100 ml of water every half hour for the next 6-8 hours. One hour after the initial fluid load, the bladder was emptied again (basal period) and the urine kept for measurement of B_2 microglobulin and N-acetyl-glucosamidinase (NAG) activity. Inulin (Kerfoot, Barnsley, U.K (N=14) or "Inutest" (Laevosan-Gesellschaft, Austria, (N=2)) and p-aminohippurate (PAH) (Merck Sharp & Dohme, U.K) were then administered intravenously for measurement of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), as described on page 50 and 173 respectively.

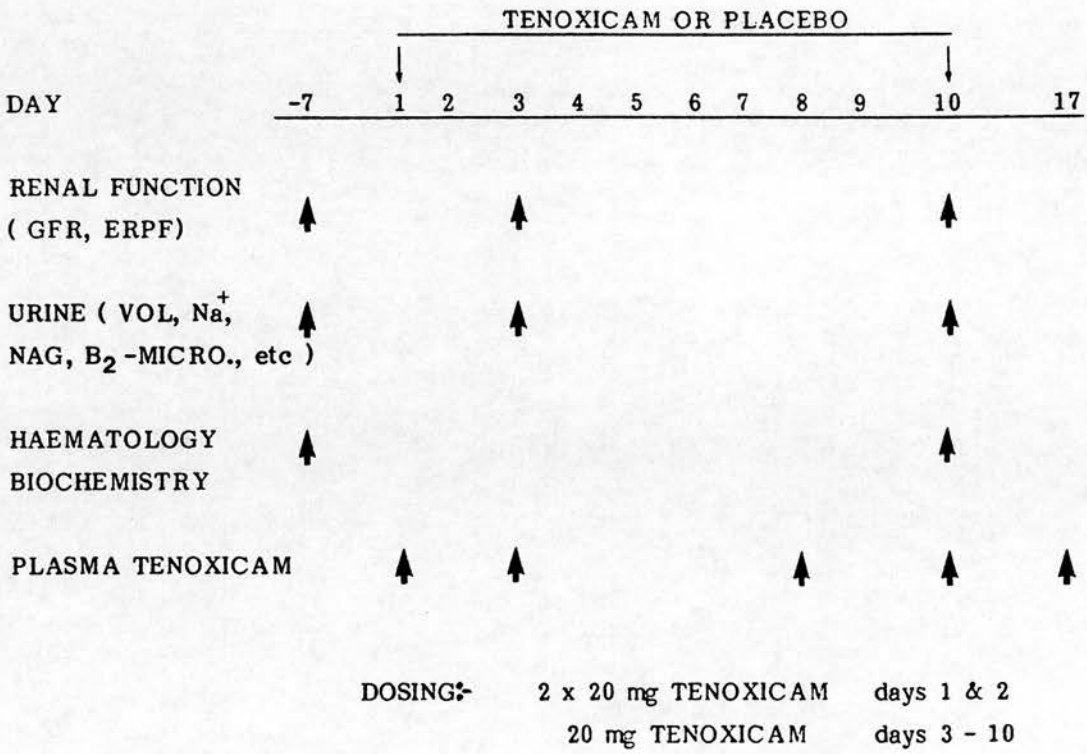
Blood was also taken before dosing on days 8 and 17 for measurement of the plasma concentrations of tenoxicam.

Sample collection

All blood samples were collected into 10 ml lithium heparin tubes, centrifuged at 1500g for 10 minutes and the plasma removed. The volume and pH of urine samples were recorded, and aliquots kept for analysis. The plasma and urine samples for measurement of inulin and creatinine were kept at 4°C, and analysed within 72 hours of collection. For the determination of PAH, samples were kept frozen at -20°C until analysis. An aliquot of the 0-24 hour urine was collected for measurement of PGE_2 and 6 keto $PGF1\alpha$ on each study day, and stored at -20°C. The pH of urine aliquots for the

Fig 4.3

Tenoxicam study design. The arrows indicate the measurements made on different days.



estimation of B_2 microglobulin was adjusted to pH 7 with 1 M sodium hydroxide.

Plasma samples for the determination of tenoxicam were stored in the dark at -20°C in plastic tubes covered with tinfoil, as tenoxicam is light sensitive.

Analytical methods

Inulin and PAH were measured as described on page 51 and 147 respectively, and the Jaffe reaction was used for determining plasma and urine creatinine by a standard autoanalyser technique (Gemstar^{TM} Electro-Nucleonics). Urine sodium and potassium were estimated by flame photometer (Corning 435), and prostaglandins PGE_2 and 6 keto $PGF1\alpha$ were determined by radioimmunoassay, as described previously (Mackay et al, 1984). Urine B_2 microglobulin was estimated by radioimmunoassay (Pharmacia, Sweden) and NAG by spectrophotofluorimetry (Leaback & Walker, 1961). Standard laboratory techniques were used for blood biochemistry and haematological screens.

Plasma tenoxicam concentrations were analysed by the Clinical Pharmacology Unit, Royal Bath Hospital, Harrogate using reverse phase HPLC (Dixon et al, 1984).

Data analysis

PAH and inulin clearance were calculated from the area under the plasma concentration-time curves (AUC), which were obtained by fitting the data to an open two compartment model using the "MULTI" computer programme (Yamaoka et al, 1981), as described in chapter two (p 53). The renal clearances of PAH and creatinine were calculated using equations 4 (p 8). i.e. The renal clearance is equal to the urinary excretion rate divided by the corresponding AUC. The clearance of PAH was determined on the 0-1 hour urine collection period only, whilst the creatinine clearance was measured for the 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 hour collection periods,

and the weighted average calculated to give the clearance for 0-8 hours. The inulin clearance was calculated as the total body clearance using equation 8 (p 23), where the total body clearance of inulin is equal to the dose of inulin injected divided by the AUC, extrapolated to infinity. The AUC extrapolated to infinity, was estimated from the 0-2 hour plasma data as described in chapter 2 (p 52). All clearance values are expressed as ml/min/1.73 m^2.

Urinary sodium and potassium excretion is expressed as mmol/24 h, prostaglandins as ng/24 h and NAG as units per mg of creatinine.

The plasma elimination half life of tenoxicam was estimated from the tenoxicam plasma concentrations on day 11 and seven days after termination of dosing, day 17, using equation 7 (p 21). The pre-dose level for day 10 was used as an estimate of the day 11 level as it was assumed that steady state concentrations were achieved and thus the pre-dose day 10 concentration would be similar to that on day 11, 24 hours after the last dose.

The results from the day before treatment started is referred to in the following text as pre, baseline or control day.

Statistical methods

The statistical significance of differences within and between the groups were determined by analysis of variance. The null hypothesis was rejected if $p < 0.05$.

RESULTS

Renal function

The mean clearances of inulin, creatinine and PAH are given in Tables 4.1 & 4.2, and the individual data are shown in Figs 4.4 & 4.6. There were no significant differences between the placebo and tenoxicam baseline values for inulin, creatinine and PAH clearances.

TABLE 4.1

0-2 h total body clearances of inulin (A) and 0-8 h renal clearances of creatinine (B) (ml/min/1.73 m^2) before (P) and on days 3 and 10 of treatment with tenoxicam or placebo in 16 healthy male subjects.

A)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	127	119	106	1	97	95	96
4	101	96	94	3	95	92	85
5	98	103	101	6	114	97	106
8	98	107	96	7	115	96	103
10	113	104	113	9	117	120	122
12	127	130	131	13	99	107	104
16	98	85	90	14	117	116	124
19	105	110	115	20	98	107	103
MEAN	108	107	106		107	104	105
±SD	13	14	14		10	10	13

B)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	119	131	126	1	104	104	113
4	99	98	100	3	116	102	101
5	125	131	130	6	113	146	170
8	126	127	117	7	115	113	109
10	119	106	123	9	116	133	127
12	135	143	125	13	117	118	119
16	103	97	107	14	119	116	113
19	137	116	124	20	112	110	118
MEAN	120	119	119		114	118	123
±SD	14	17	10		5	15	22
Mean data after omitting subject No 6's data					114	114	116

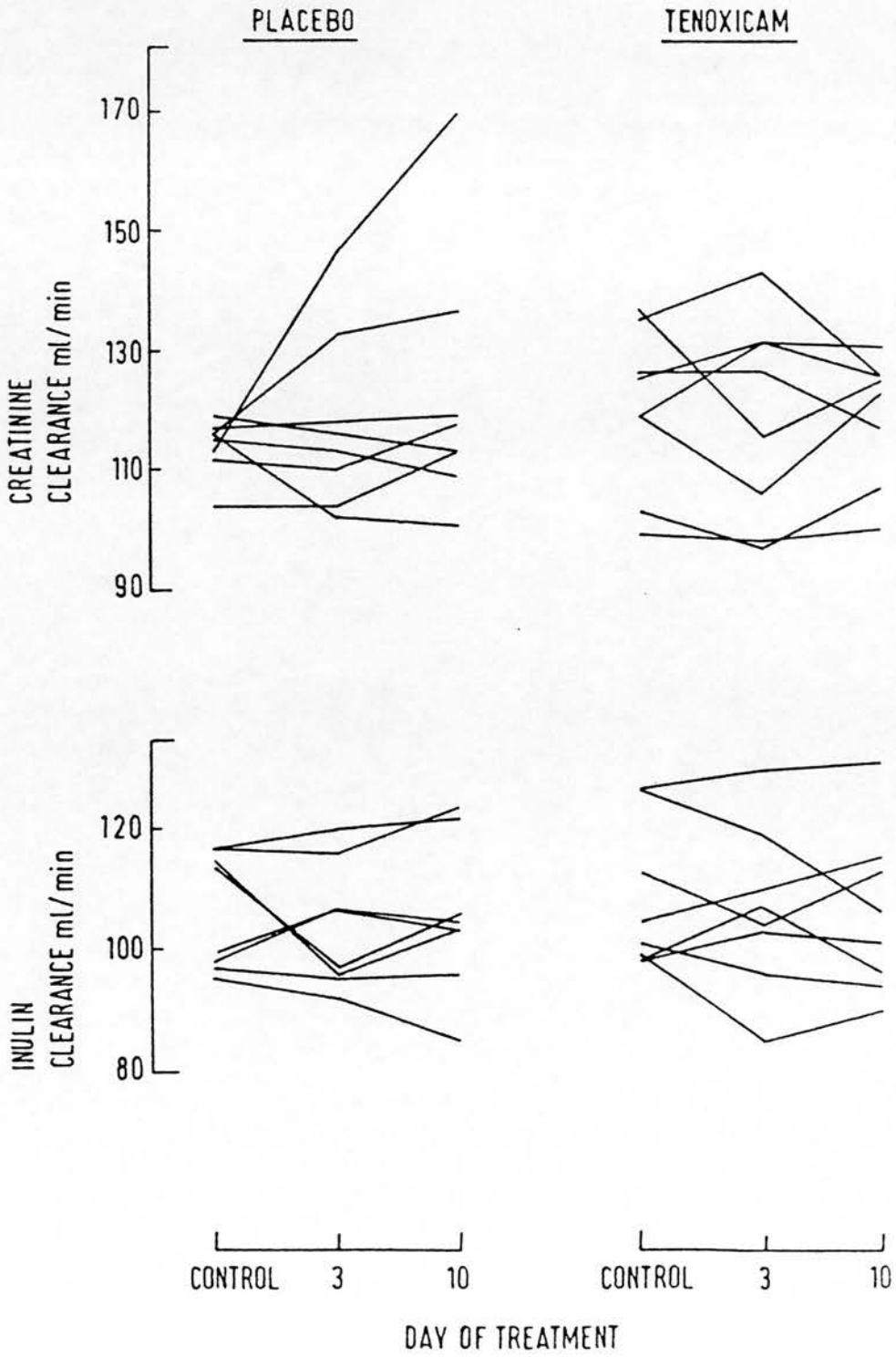
TABLE 4.2

The 0-1 h renal clearance of PAH (ml/min/1.73 m^2) before (P) and on days 3 and 10 of treatment with tenoxicam or placebo in 16 healthy male subjects.

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	619	723	678	1	463	533	492
4	495	447	439	3	526	414	611
5	528	519	597	6	533	578	360
8	649	643	711	7	597	514	545
10	545	489	623	9	846	791	818
12	621	750	540	13	605	728	665
16	449	434	416	14	623	735	613
19	513	428	498	20	536	573	510
MEAN	552	554	563		591	608	578
±SD	70	132	108		115	130	135

Fig 4.4

Individual 0-2 h total body clearances of inulin (bottom) and 0-8 h renal clearances of creatinine (top) in 16 healthy males, before (control) and on days 3 and 10 of treatment with placebo or tenoxicam. Clearances expressed as ml/min/1.73 m^2.



The mean total body clearances of inulin were 108, 107, and 106 ml/min/1.73 m^2 in the tenoxicam group, and 107, 104 and 105 ml/min/1.73 m^2 in the placebo group before, and on days three and ten of treatment (Fig. 4.5). Thus, tenoxicam had no significant effect on the glomerular filtration rate.

Similarly, there was no significant change in the creatinine clearance. The mean creatinine clearances were 120, 119, and 119 ml/min/1.73 m^2 for the tenoxicam group, and 114, 118 and 123 ml/min/1.73 m^2 for the placebo group before, and on days three and ten of treatment (Fig. 4.5). The placebo group showed a rise in mean creatinine clearance over the treatment period, but this was attributable to subject No 6 whose creatinine clearance rose markedly. On removal of this subject's data, no rise is seen (Fig. 4.5).

There was considerable inter individual variation in the PAH clearance (Fig. 4.6), but there were no significant differences between the placebo and tenoxicam groups. The mean PAH clearances were 552, 554, and 563 ml/min/1.73 m^2 in the tenoxicam group and 591, 608 and 577 ml/min/1.73 m^2 in the placebo group before, and on days three and ten of treatment (Fig. 4.6). There was no significant change in the mean PAH clearance over the treatment period.

Urine volume and electrolyte excretion

The individual and mean values for the 24 hour urinary excretion of sodium and potassium, and urine volume are given in Tables 4.3 & 4.4.

There were no statistically significant changes in sodium excretion or urine volume in the placebo or tenoxicam groups (Fig. 4.7). The mean urinary potassium excretion fell progressively in the tenoxicam group, but this trend was not statistically significant (Fig. 4.7).

Fig 4.5

Mean 0-2 h total body clearance of inulin (bottom) and 0-8 h renal clearances of creatinine (top) in 16 healthy males, before (control) and on days 3 and 10 of treatment with placebo or tenoxicam. (--0--) is the mean clearance, omitting data from subject No 6). Clearances are expressed as ml/min/1.73 m^2 and bars = $\pm$ SD.

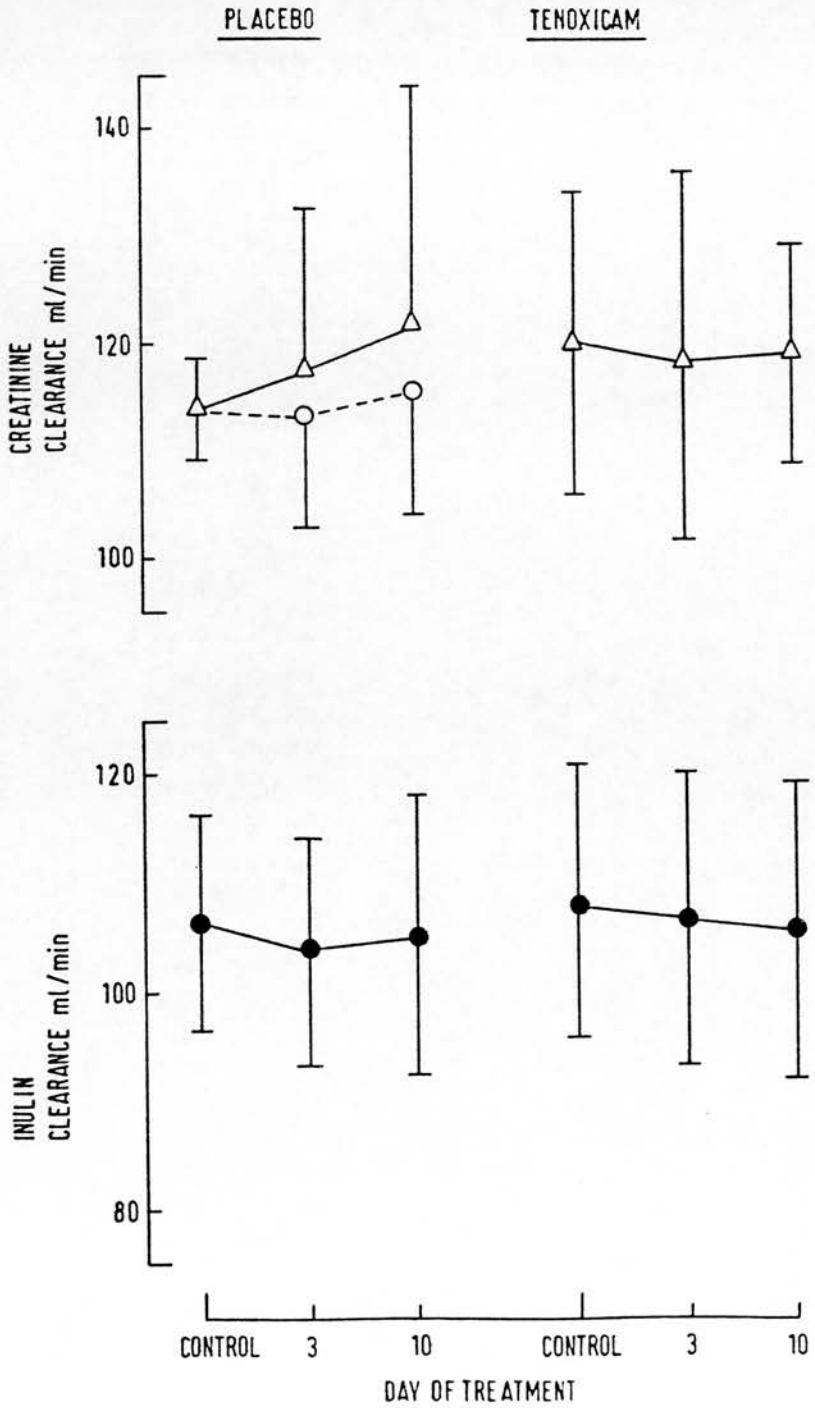


Fig 4.6

Mean (top) and individual (bottom) 0-1 h renal clearances of PAH in 16 healthy males, before (control) and on days 3 and 10 of treatment with placebo or tenoxicam. Bars = ± SD. Clearances are expressed as ml/min/1.73 m^2

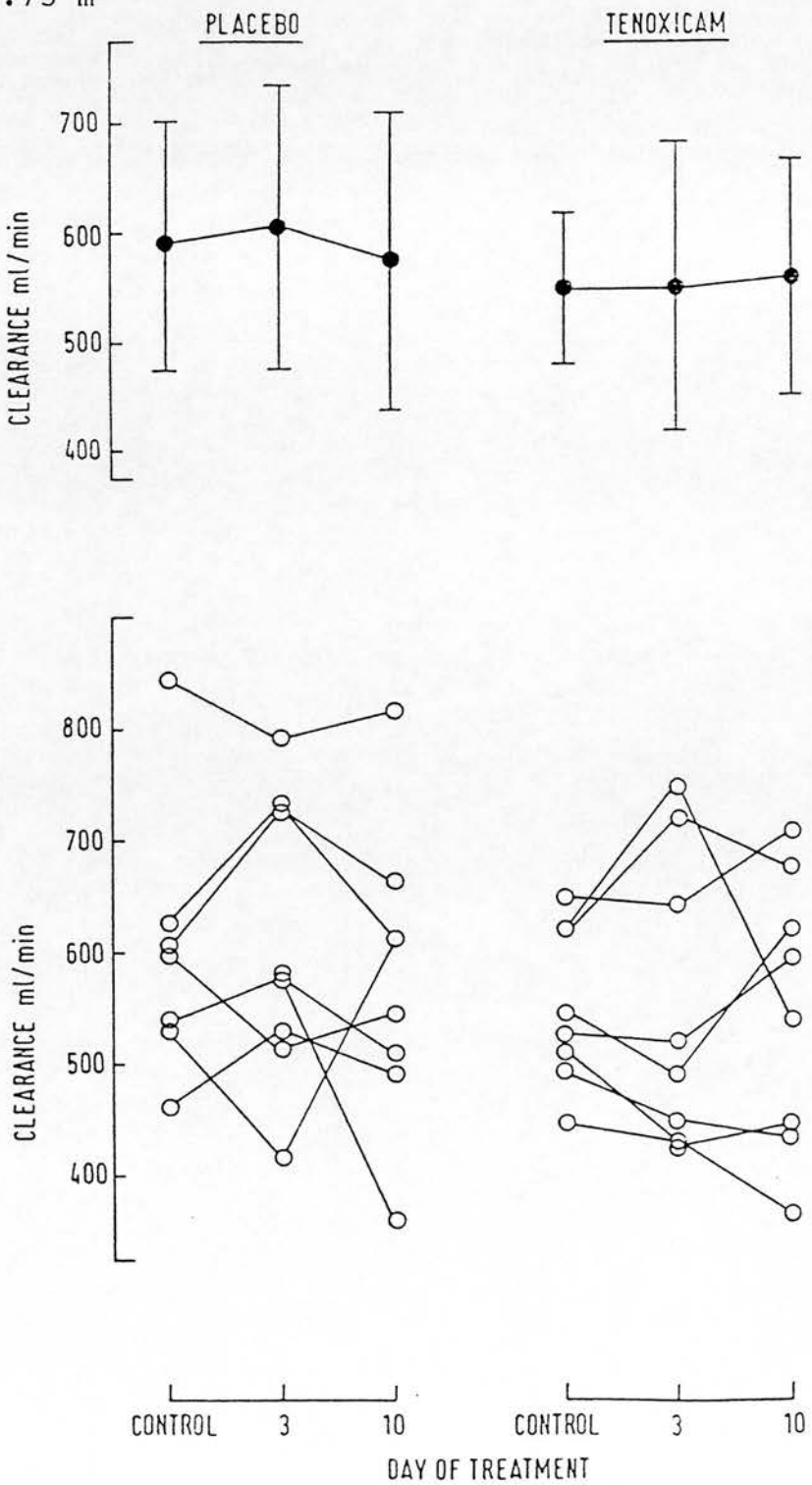


TABLE 4.3

24 H urinary sodium (A) and potassium (B) excretion (mmol/24h) before (P) and on days 3 and 10 of treatment with tenoxicam or placebo in 16 healthy male subjects.

A)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	189	187	121	1	177	244	90
4	87	99	102	3	230	164	127
5	174	145	129	6	135	165	171
8	190	245	166	7	163	81	132
10	188	179	185	9	247	195	190
12	123	240	203	13	113	70	115
16	95	64	71	14	119	195	196
19	161	154	184	20	131	79	197
MEAN	151	164	145		164	149	152
±SD	43	63	46		51	65	41

B)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	55	65	46	1	82	76	39
4	65	40	47	3	37	58	39
5	70	86	57	6	86	55	91
8	86	103	102	7	47	52	49
10	64	68	60	9	71	59	72
12	137	64	48	13	84	39	49
16	31	41	39	14	43	60	75
19	80	58	53	20	58	50	62
MEAN	73	66	57		64	56	59
±SD	31	21	20		20	11	19

TABLE 4.4

24 H urine volume (ml) before (P) and on days 3 and 10 of treatment with tenoxicam or placebo in 16 healthy male subjects.

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	3502	4334	2614	1	3255	3288	2952
4	3229	3229	3218	3	2653	3391	2522
5	3453	3795	3227	6	3090	3297	3186
8	3542	3580	3197	7	2973	2298	3092
10	2765	2633	3140	9	3065	2901	2906
12	3071	3488	3751	13	3338	2327	2186
16	2546	1864	2092	14	3559	3930	3676
19	2881	3091	2685	20	3113	2506	2756
MEAN	3124	3242	2991		3131	2992	2910
±SD	371	753	506		268	584	447

TABLE 4.5

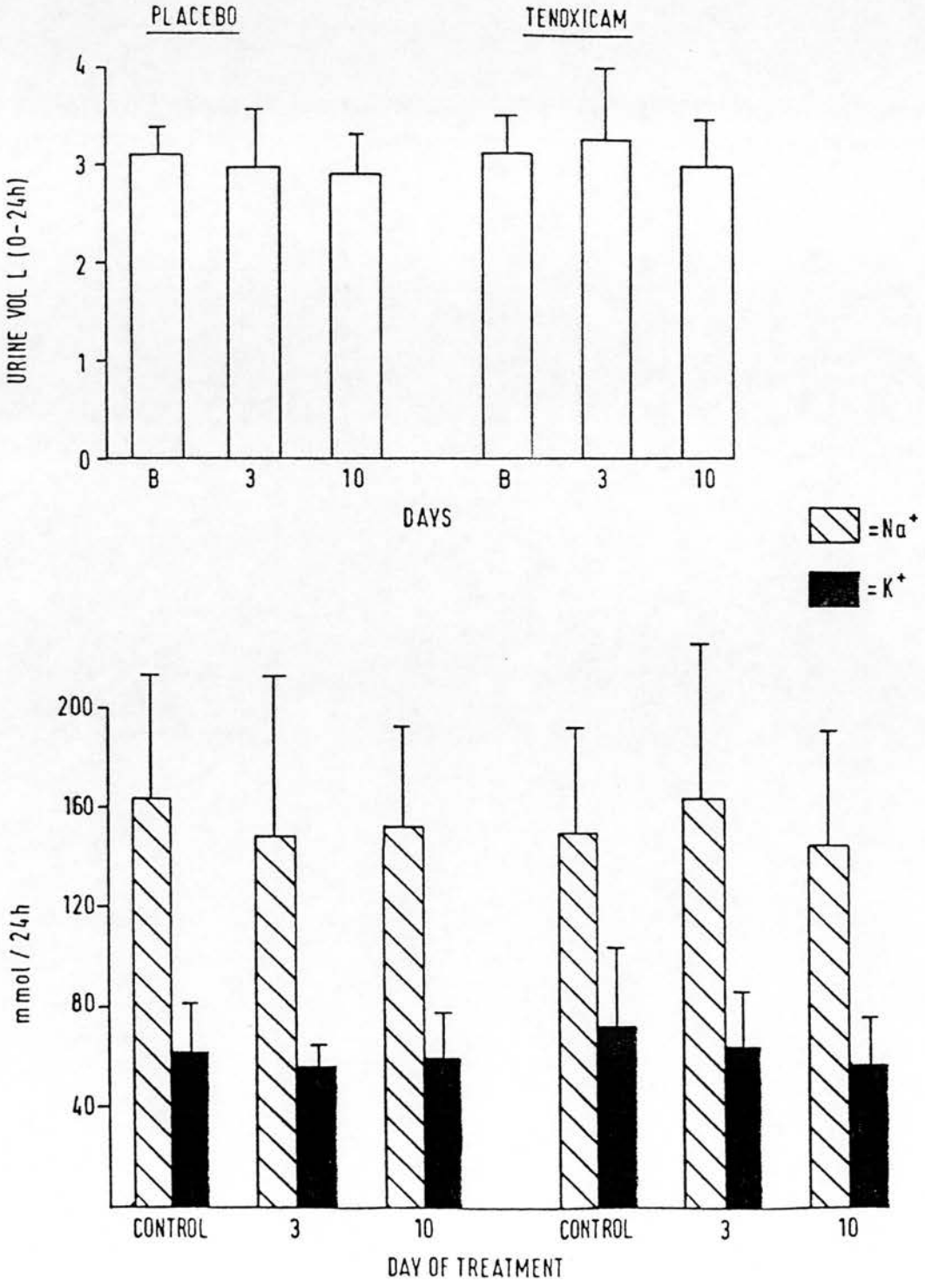
0-1 H urinary NAG excretion before (P) and on days 3 and 10 of treatment with tenoxicam or placebo in 16 healthy male subjects.

URINARY NAG EXCRETION (UNITS / MG CREATININE)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	48	45	70	1	72	82	51
4	163	273	134	3	92	129	92
5	43	44	35	6	227	139	191
8	68	68	99	7	112	144	122
10	138	82	121	9	80	73	71
12	264	131	288	13	109	97	115
16	50	72	75	14	75	107	92
19	126	75	84	20	136	133	132
MEAN	113	99	113		113	113	108
±SD	77	75	77		51	27	43

Fig 4.7

Mean 24 hour urine volume (top) and sodium and potassium excretion (bottom) in 16 healthy males, before (B or control) and on days 3 and 10 of treatment with placebo or tenoxicam. Bars = SD.



Urine B_2 microglobulin and NAG concentrations.

Urinary B_2 microglobulin concentration was within normal limits (<0.3 mg/l) throughout the study in all subjects except in two cases (one in each group), where the concentration rose to 0.4 mg/l. The mean urinary NAG excretion did not change during the treatment period in either group (Table 4.5).

Prostaglandins

The urinary excretion of prostaglandins PGE_2 and 6 keto PGF1α was very variable in both treatment groups (Table 4.6 A & B). The marked fluctuations in prostaglandin output precluded any meaningful statistical comparisons between the placebo and tenoxicam groups.

Plasma tenoxicam concentrations

The plasma concentrations of tenoxicam are given in Table 4.7. The mean plasma concentrations of tenoxicam were 4.68 $\pm$ 0.92, 6.58 $\pm$ 1.56, 6.53 $\pm$ 1.87, and 2.50 $\pm$ 1.61 mg/l on days 3, 8, 10, and 17. In six subjects, the plasma concentrations remained relatively constant from day 8 to day 10, but in two (Nos 2 and 19) concentrations were still rising (Fig. 4.8). By day 17 (seven days after stopping the drug), the plasma concentrations were less than on day 3, except in subject No 19. The mean plasma elimination half life as calculated from the day 11 and 17 value was 105.5 $\pm$ 50.1 hours. This was exceptionally long in subject No 19, with a half life of 207.9 hours. The individual half lives are given in Table 4.7.

Adverse effects

Tenoxicam was well tolerated by all except subject No 19, who experienced persistent, intermittent epigastric and central abdominal pain. It started on the fourth day of treatment, and lasted for two weeks ranging in severity from mild to severe. He completed

TABLE 4.6

24 H urinary excretion (ng/24 h) of PGE_2 (A) and 6 keto PGF1α (B) before and on days 3 and 10 of treatment with tenoxicam or placebo in 16 healthy male subjects.

A)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	9890	3710	1281	1	570	612	266
4	5505	2005	3369	3	525	482	525
5	13311	1158	284	6	556	679	17924
8	900	695	1017	7	898	499	618
10	17397	5100	21324	9	18298	5437	3888
12	989	970	259	13	1008	251	643
16	812	725	318	14	1730	1662	890
19	1026	3743	781	20	>20235	383	1560
MEAN	6229	2263	3579		5478	1251	3289
±SD	6558	1695	7242		8535	1746	6026

B)

	TENOXICAM				PLACEBO		
SUBJECT	P	3	10	SUBJECT	P	3	10
2	1684	1386	614	1	680	802	543
4	1450	1201	1210	3	446	536	446
5	1495	1450	600	6	717	692	2444
8	1031	795	1029	7	612	464	473
10	1366	487	1193	9	3307	2211	1485
12	1766	586	521	13	651	342	514
16	535	296	418	14	456	495	467
19	274	291	618	20	3054	356	659
MEAN	1200	812	775		1240	737	879
±SD	543	476	317		1203	616	720

TABLE 4.7

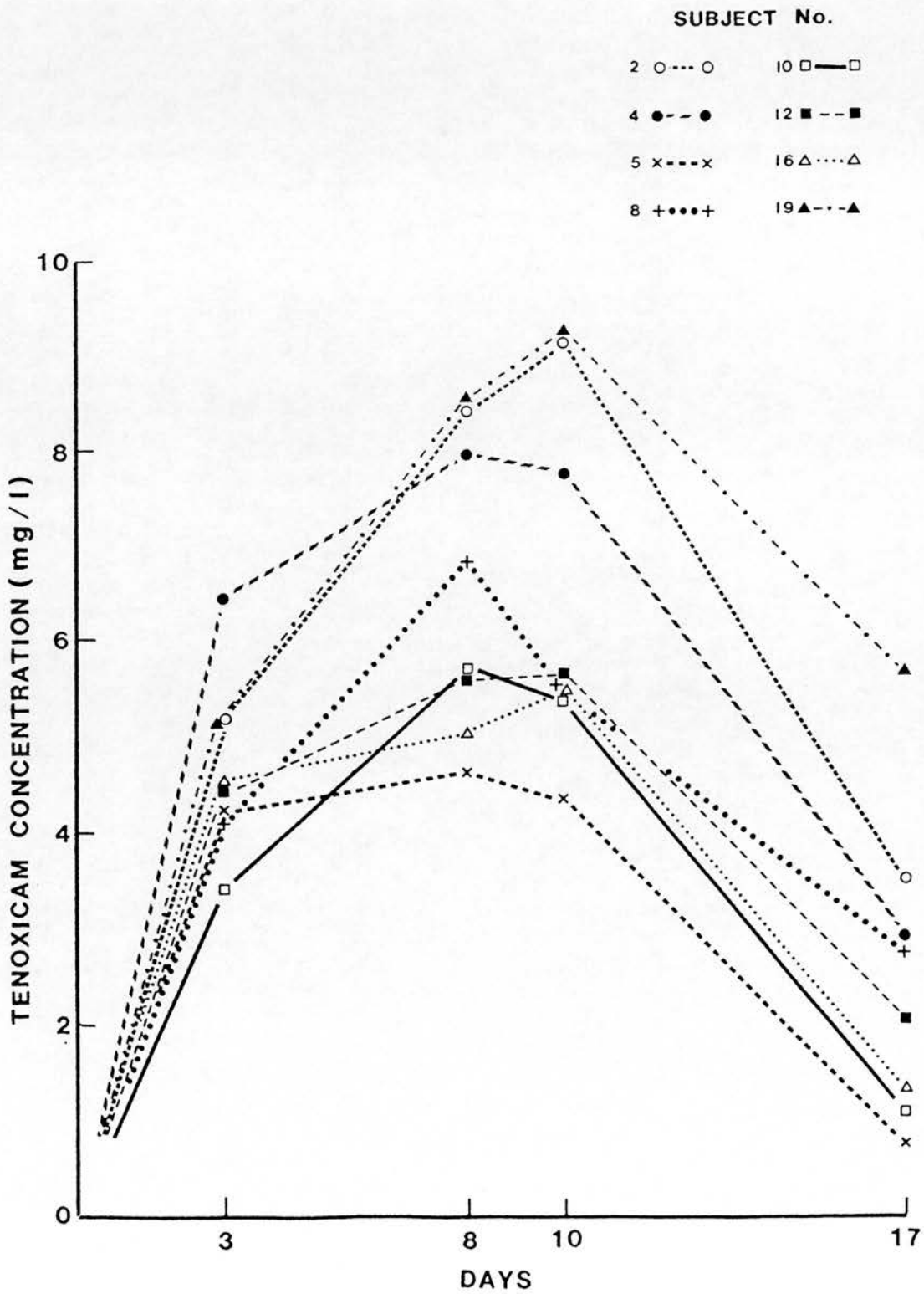
Plasma tenoxicam concentrations on days 3, 8, and 10 of treatment in 8 healthy male subjects, and 7 days after treatment was stopped. The plasma elimination half life ($t_{\frac{1}{2}}$) is also shown.

	PLASMA TENOXICAM CONCENTRATIONS (mg/l)				
	DAY				
SUBJECT	3	8	10	17	*$t_{\frac{1}{2}}$ (h)
2	5.19	8.38	9.12	3.52	104.8
4	6.44	7.95	7.74	2.91	102.0
5	4.21	4.63	4.35	0.74	56.4
8	4.11	6.82	5.51	2.74	142.9
10	3.37	5.67	5.35	1.04	60.9
12	4.42	5.62	5.60	2.04	98.8
16	4.52	5.01	5.41	1.31	70.4
19	5.15	8.53	9.18	5.68	207.9
MEAN	4.68	6.58	6.53	2.50	105.5
±SD	0.92	1.56	1.87	1.61	50.1

* Calculated from day 11 and 17 data

Fig 4.8

Individual plasma tenoxicam plasma concentrations.



the ten day course of tenoxicam, and the symptoms were probably related to tenoxicam. No similar symptoms were encountered in the placebo group.

DISCUSSION

Under the conditions of this study, tenoxicam did not have a demonstrable effect on GFR, ERPF or electrolyte excretion in healthy subjects. Steady state plasma concentrations were achieved in the majority of subjects by the tenth day of treatment. The mean concentration on day ten was 6.53 mg/l, and the mean plasma half life was 105.5 $\pm$ 50 hours. This is greater than the reported average half life of tenoxicam (Gonzalez & Todd, 1987). However, the half life was considerably longer in subject No 19, and he was the only subject to suffer adverse effects. The gastrointestinal side effects of non-steroidal anti-inflammatory drugs have been well documented (Nickander et al, 1979).

The lack of effect of tenoxicam on renal function in these healthy individuals is not surprising, since similar negative findings have been reported with other non-steroidal anti-inflammatory drugs (indomethacin, ibuprofen, asprin) under similar conditions, including those of the same class (eg piroxicam) (Darragh, 1985; Dibona, 1986; Patrono & Dunn, 1987). However, in other studies reduced GFR, ERPF and sodium excretion have been noted in healthy subjects who are sodium deplete (Clive & Stoff, 1984; Raymond & Lifschitz, 1986).

Non-steroidal anti-inflammatory drugs are thought to mediate most of their therapeutic and adverse effects through inhibition of prostaglandin synthesis, and in the kidney this can result in reduced RBF and GFR, with adverse effects on renal function under certain conditions (Nickander et al, 1979; Clive & Stoff, 1984). Renal prostaglandins are synthesised, act and are degraded within the kidney. The major renal prostaglandins are the powerful vasodilators prostacyclin (PGI_2) and PGE_2, and thromboxane A2 which is a vasoconstrictor. PGI_2 and thromboxane are formed in the glomerulus,

arterioles and juxtaglomerular apparatus (PGI_2 only), while PGE_2 is formed within the medullary interstitial cells and collecting tubules. One of the major roles of the prostaglandins is the maintenance of renal cortical and medullary function in patients with kidney, liver and heart disease (Dunn, 1984; Patrono, 1986).

Experimental and clinical evidence has shown that with increased amounts of circulating vasoconstrictors (eg angiotensin II, vasopressin and catecholamine), the renal circulation depends on a dynamic balance between vasoconstriction and vasodilation. The prostaglandins PGI_2 and PGE_2 maintain renal vasodilation by reducing the effects of the vasoconstrictors on pre- and postglomerular arterioles, and also by relaxing the mesangium, which preserves the glomerular filtration surface area. In this way, the renal blood flow and GFR are maintained (Dunn, 1984). This protective mechanism is of particular importance in patients with reduced renal perfusion caused by cirrhosis with ascites, diuretic induced volume and sodium depletion, congestive cardiac failure and nephrotic syndrome, as well as in the elderly (Prescott, 1982b). The use of non-steroidal anti-inflammatory drugs in these situations, leads to inhibition of renal prostaglandin synthesis and a decrease in renal blood flow and GFR. This may cause renal ischaemia and lead to acute or chronic renal failure (Carmichael & Shankel, 1985). The acute effects are usually reversible on stopping the non-steroidal anti-inflammatory drug.

In normal healthy individuals, renal blood flow is not dependent upon endogenous prostaglandin synthesis, and non-steroidal anti-inflammatory drugs have little, or no effect on renal function as other regulatory mechanisms (eg adrenergic tone, renin secretion, and dopamine) are also unstressed and therefore, compensate when prostaglandins are inhibited (Patrono & Dunn,

1987).

Inhibition of prostaglandin synthesis also has effects on urinary electrolyte and water excretion. This is due in part to a reduction in renal blood flow, and thus the filtered load, and also to potentiation of the effect of anti-diuretic hormone (ADH) (Clive & Stoff, 1984; Raymond & Lifschitz, 1986). Renal prostaglandins also promote sodium and water excretion by a direct action on the renal tubules, and their inhibition may reduce sodium and water excretion without necessarily changing the ERPF or GFR (Haylor, 1980). No significant effects on sodium or water excretion were seen in the present study, but the subjects were fluid loaded on the day of study, and had been on tenoxicam for two days before the first observations were made. A small initial transient effect could have been missed.

Retention of potassium with hyperkalaemia has also been reported after administration of non-steroidal anti-inflammatory drugs. This is thought to be mediated through inhibition of renin release, which in turn reduces circulating aldosterone (Staszewska-Barczak, 1978). PGI_2 has been reported to be involved in the release of renin (Clive & Stoff, 1984). No significant changes in 24 hour urinary potassium excretion were seen, in the present study.

The long term use of non-steroidal anti-inflammatory drugs may cause chronic renal injury including papillary necrosis and chronic interstitial nephritis (Adams et al, 1986; Prescott, 1982b). The proximal tubule has a high metabolic activity, and is particularly vulnerable to nephrotoxicity. Toxic effects on the proximal tubules can be detected by abnormal urinary excretion of compounds which undergo tubular transport at this site, and by the release of specific enzymes from tubular cells. Increased excretion of B_2 microglobulin reflects impaired tubular reabsorption of this low molecular

weight protein, while excessive urine NAG activity, indicates leakage from damaged tubular cells (Prescott, 1982a). No increase in the excretion of either B_2 microglobulin or NAG was detected in this study.

Measurement of the urinary excretion of prostaglandins and their metabolites, is thought to reflect renal prostaglandin synthesis (Patrono, 1986). With the possible exception of sulindac, all non-steroidal anti-inflammatory drugs reduce urinary prostaglandin excretion by at least 50 % when administered in full anti-inflammatory doses (Patrono & Dunn, 1987). Urine prostaglandin E_2 is primarily derived from medullary sites, and the metabolite of prostacyclin, 6-keto PGF1α, from the cortex (Patrono, 1986). Unfortunately, the variation in the urinary excretion of PGE_2 and 6-keto PGF1α in the present study was such, that no conclusions could be drawn concerning the effects of tenoxicam. The reason for this large variability is probably contamination with prostatic fluid. This is common in men, and urinary prostaglandin measurements in men are probably uninterpretable (Patrono & Dunn, 1987).

SUMMARY

The short term administration of a new non-steroidal anti-inflammatory drug, tenoxicam in healthy hydrated males, had no significant effects on renal function under these conditions, but inhibition of renal prostaglandin synthesis could not be demonstrated. However, adverse renal effects in patients predisposed to nephrotoxicity and the elderly cannot be excluded.

CHAPTER FIVE

FINAL DISCUSSION AND CONCLUSIONS

DISCUSSION

A single injection method for measuring glomerular filtration rate (GFR) and renal plasma flow (RPF) has been examined, using appropriate pharmacokinetic analysis. The use of single injection methods for measuring GFR and RPF are today widely accepted as accurate and reproducible, if appropriate pharmacokinetic methods of analysis are used (Cohen, 1974; Brochner-Mortensen, 1985). The standard methods for measuring GFR and RPF by constant infusion (Schuster & Seldin, 1985) are inconvenient for both patient and investigator. Short accurately timed urine collections are needed, the patient is immobilised for several periods, and forced diuresis or catheterization are required for accurate urine collections. These techniques are therefore not practicable for routine clinical use (Kampmann & Molholm-Hansen, 1981). One important advantage of the single injection method is that urine collection is not necessary. This eliminates the greatest source of error and inconvenience in constant infusion methods. In addition there is no need to wait for steady state to be reached.

Inulin and p-aminohippuric acid (PAH) are the standard substances for estimation of the GFR and RPF respectively (Schuster & Seldin, 1985). Their clearances are believed to be constant at plasma concentrations achieved in the present studies and independent of the urine flow rate, but the clearance of PAH decreases above 80 mg/l due to saturation of the renal transport system (Smith, 1951). These properties should make inulin and PAH ideal for use in single injection methods, but most data related to their clearance refers to the standard constant infusion methods. The independence of the PAH and inulin clearance of their respective plasma concentrations is a major assumption, which is made in their use as reference compounds.

The results from the present studies clearly show that this assumption does not hold at low plasma concentrations. The clearance of both inulin and PAH were found to be significantly lower at low than at high plasma concentrations, regardless of whether the plasma concentrations were at steady state or falling. In the case of inulin, the renal clearance fell in subjects with both normal and impaired renal function. For both compounds the significant changes in clearance were related to plasma concentration. To elucidate, the possible mechanisms involved it is necessary to remember that the renal clearance of any substance is the resultant of 4 processes (Arvidsson, 1982):-

1) Glomerular filtration.
2) Active secretion into tubular fluid.
3) Active reabsorption from tubular fluid.
4) Renal Metabolism.

The use of the renal clearance of inulin as a measure of the GFR over any period of time, depends on the assumption that it reflects glomerular filtration only (Smith, 1951). In the case of PAH, there is both filtration and active proximal tubular secretion with 90 % extraction in one passage through the kidney, so, it is ideal for measurement of the effective renal plasma flow (Smith et al, 1945). However, the results of the present studies show, that there are fundamental changes in the clearance of both PAH and inulin as the plasma concentration declines. In the case of inulin the renal clearance falls progressively as the plasma concentration decreases below 100 mg/l in subjects with normal renal function. This threshold level is higher in patients with renal impairment. The decrease in PAH clearance becomes significant when plasma concentrations fall below 20 mg/l.

These changes in the clearances of inulin and PAH cannot be explained by effects such as delay time

errors, plasma protein binding, uptake into erythrocytes, arterial-venous differences, selective filtration of the low molecular weight polymers of inulin, or time itself, as shown by the "step up" and "step down" studies. Similar results have been reported by Smith, 1951; Newman et al, 1949; Ferguson et al, 1950; Mogensen, 1968. The observed falls in clearances were greater following a single injection than during constant infusion. Some of the above effects could have contribute to some extent to the changes following a single injection, as they are more likely to occur with declining plasma concentrations than at steady state (Pihl, 1973).

With inulin, three observations must be taken into account, in relation to possible mechanisms for the decreasing clearance:-

1) The renal clearance of inulin was less than the glomerular filtration rate at low plasma concentrations, since there was no corresponding fall in the simultaneously determined creatinine clearance. If the clearance of any substance is less than the glomerular filtration rate, tubular reabsorption is possible (Rowland & Tozer, 1980).

2) The total body clearance of inulin is significantly greater than the renal clearance of inulin after a single injection. Significant extrarenal clearance of inulin is unlikely since it is quantitatively recovered in the urine. Underestimation of the area under the plasma concentration-time curve following a single injection,could account for the difference, (Rehling et al, 1984) and this may have occurred, since it is impossible to define the early part of the AUC accurately. However, the same differences in clearance were seen during the constant infusion studies.

3) A plot of the urinary excretion rate of inulin against the plasma concentration shows a negative

deviation from the origin. This indicates that the observed rate of inulin excretion lags behind the plasma concentration (Tucker, 1981). Such an effect could be explained by the delay time, a progressive increase in the time taken for inulin to pass through the kidney, or its retention within the renal tract because of increasing residual urine as the flow rate declines. These mechanisms seem unlikely to have caused the decreased clearance of inulin, as they cannot account for the changes in inulin clearance during the "step up" and "step down" constant infusion studies. Under these conditions the high urine flow rates minimise the effects of delay time and errors caused by residual urine (Pihl, 1973). Perhaps the most likely explanation is saturable tubular reabsorption.

This possibility was first raised by Ferguson et al, (1950), but similar studies by Laake, (1954), and Kennedy & Kleh, (1953) gave different results. These authors concluded that there was no evidence to support tubular reabsorption and this appears to be confirmed by micropuncture studies in the rat (Harris et al, 1974). Uptake of inulin into the renal tubules has been reported to occur in animals (Balint & Forgacs, 1958; Gayer et al, 1961) and the results of the present studies are entirely consistent with low capacity saturable tubular reabsorption of inulin, which only becomes evident at low plasma concentrations. Further studies are required to elucidate the kinetics of this reabsorption process.

The circumstances of the decrease in the renal clearance of PAH are different since it is metabolised to AcPAH. The following evidence points to a possible mechanism for the decline in PAH clearance at low plasma concentrations:-

1) As the renal clearance of PAH falls with decreasing plasma concentrations, the proportion of the

dose recovered in the urine as AcPAH increases. The opposite occurs as the plasma concentrations of PAH increase.

2) The total body clearance of PAH is significantly greater than the renal clearance following single injection and constant infusion studies. This difference can be accounted for entirely by metabolism as the administered dose is quantitatively recovered in the urine as PAH and AcPAH.

3) The renal clearance of AcPAH is greater than the renal clearance of PAH, after intravenous administration of PAH. The clearance of any substance cannot be greater than the renal blood flow, unless it is synthesised within the kidney, or unless the measured plasma concentration differs significantly from the whole blood concentration, because of uptake into erythrocytes (Smith, 1951). At low plasma concentrations of PAH, the apparent clearance of AcPAH is greater than would be expected if AcPAH was given alone (Smith et al, 1945; Newman et al, 1949). This is consistent with renal metabolism of PAH (Tucker, 1981).

4) The total body clearance of PAH does not change during constant infusion, but the difference between the total body and the corresponding renal clearance, diminishes at high plasma concentrations of PAH. This suggests that the change in PAH clearance is related to its metabolism by the kidney.

5) The renal clearance of total PAH + AcPAH (as PAH equivalents) is relatively constant at different plasma concentrations of PAH and it is significantly greater than the renal clearance of PAH. Again this difference diminishes as the plasma concentration of PAH increases.

Taken together these observations cannot be explained by a change in the renal extraction of PAH or in its extrarenal metabolism. The findings could conceivably be explained in part by bidirectional renal

tubular transport of PAH as has been reported for the cephalosporin, cephapirin. This also shows a similar decline in renal clearance as its plasma concentrations decline (Arvidsson, 1982). There is indeed evidence for the tubular reabsorption of PAH "in vitro" and "in vivo", in animals (Cho & Cafruny, 1970; Moller & Sheikh, 1983). However, as the clearance of PAH is much greater than the glomerular filtration rate, secretion must still be the major excretory mechanism at low plasma concentrations (Rowland & Tozer, 1980). Saturable tubular reabsorption of PAH at low concentrations is inconsistent with the fact that the renal clearance of AcPAH, is significantly greater than the renal clearance of PAH, even at high plasma concentrations, under these conditions, the renal clearance of PAH is considered to be an accurate measure of the renal blood flow (Schuster & Seldin, 1985), and it cannot exceed it. The renal clearance of AcPAH increases significantly as the plasma concentrations of PAH decline and this could be due to saturable concentration-dependant renal acetylation of PAH.

Renal acetylation of PAH has been reported "in vivo" in animals (Setchell & Blanch, 1961), "in vitro" in man (Frindt & Vial, 1968) and possibly "in vivo" in man (Newman et al, 1949; Grindt et al, 1974). The metabolism of PAH has been reported not to effect its renal clearance (Smith, 1951; Pearson, 1979). This may be true at high plasma concentrations, if the renal acetylation becomes saturated, but this appears not to be the case at low plasma concentrations of PAH (below 20 mg/l). The renal tubular transport of PAH becomes saturated at high plasma concentrations, causing a depression in its renal clearance (Schuster & Seldin, 1985) and therefore if PAH is to be used as a measure of the RPF, the plasma concentrations must be maintained, in the range in which both these effects are minimal. Thus the renal clearance

of PAH is not an accurate measure of the renal plasma flow at low plasma concentrations and this is probably due to significant saturable renal acetylation. This holds for both single injection and constant infusion methods.

Are the renal clearances of inulin and PAH valid measures of GFR and RPF following single intravenous administration ?

The single injection technique is clearly applicable to inulin, as its total body and renal clearances are similar to those measured by the constant infusion and they are significantly more reproducible, in repeated studies. However, the plasma concentrations of inulin must be above the levels where inulin reabsorption becomes significant. In the present study, this threshold level was about 100 mg/l and this is reached approximately 2 hours following a dose of 70 mg/Kg, in subjects with normal renal function.

Today the endogenous creatinine clearance is the most widely used measure of the GFR; in most hospitals. An accurate 24 hour urine collection is usually required, but creatinine clearance can also be estimated from the plasma creatinine concentration, using a nomogram based on age, sex and body weight (Brochner-Mortensen, 1985). However, the renal clearance of creatinine is an overestimate of the GFR because of tubular secretion and this increases as the renal function declines (Bauer et al, 1982). Thus, the creatinine clearance is not an ideal measure of the GFR. Cr^{51} EDTA and I^{131} iothalamate have also been used but the former underestimates the renal clearance of inulin by 5-15 % (Brochner-Mortensen, 1985) and iothalamate is believed to undergo extrarenal clearance and renal tubular secretion (Odlind et al, 1985; Prueksaritanont et al, 1986). These radiolabelled compounds are probably not as accurate as inulin for measurement of the GFR and

there are inherent dangers with the use of radioactive materials, especially if repeated estimates are required. This is a particular problem in children and here the inulin single injection technique has been used successfully (Muller-Suur et al, 1983).

The results of the present studies show that in adults, the inulin single injection method is as accurate and more reproducible than the constant infusion method. It is also comfortable for the patient, and fluid loading, catheterisation and prolonged immobilization are not required. It is an ideal technique for the single estimation of GFR, but if dynamic studies are required, the constant infusion method is probably still the best method. With the single injection technique, the numerous blood samples required to define the plasma concentration-time curve, are a drawback.

The validity of the renal clearance of PAH, following single injection as a measure of the RPF, is more complicated since it is acetylated both renally and extrarenally. Therefore, the total body clearance may be an overestimate or the renal clearance may be an underestimate of the true RPF. The extrarenal metabolism influences the total body clearance, while the renal metabolism influences the renal clearance. It is therefore necessary to know the contribution of each process. In the present studies, it appears that the renal metabolism of PAH becomes saturated and is insignificant at high plasma concentrations. Therefore, in constant infusion studies with plasma concentrations of the order of 30 mg/l the renal clearance of PAH is a more accurate measure of the RPF than the total body clearance, as the intrarenal metabolism is probably insignificant. The renal clearance of PAH during the first hour following a single injection in the present study was of the same order as that achieved during constant infusion. This

suggests that after a single injection, the renal clearance of PAH is not initially influenced to any great extent by renal metabolism. Thus under the conditions of this study the single injection method gives an estimate of the renal clearance of PAH, which is probably a close approximation to the ERPF, when sampling is limited to the first hour. The single injection method of measuring the renal clearance of PAH has the advantages described previously and, as it is very quick, repeated estimates can be made on the same day. Although it is a safe and simple method, urine collections are required and at low plasma concentrations, major errors are likely, because of the renal metabolism.

At low plasma concentrations of PAH, it is not possible to correct the total body clearance for extrarenal clearance, because the contribution of the renal acetylation is unknown. However, provided the plasma concentrations are kept above the point where the renal metabolism becomes significant, it should be possible to apply the appropriate correction factor to the total body clearance. Thus, to eliminate the errors involved in achieving accurate urine collections, infusion to steady state plus a correction factor for extrarenal PAH metabolism, would probably be the best method for estimating the ERPF. This method would still require urine collections, but only to acertain the fraction of the dose infused which is converted extrarenally to AcPAH.

The effective renal plasma flow has been measured using the single injection method before, with radiolabelled compounds such as, I^{131} diodrast and Hippuran (Pihl, 1973; Pearson, 1979). The results with these substances are not as accurate as with PAH, and at low plasma concentrations, their clearances also decline (Block & Burrows, 1960; Pihl, 1973). With radioactive

iodine it is necessary to pre-treat the patient with potassium iodide to inhibit the uptake of radioactive iodine into the thyroid (Pearson, 1979). In comparison with these other test compounds, the use of PAH by single injection methods may be no worse. However, it is not ideal and there is a need for better agents for measuring the renal plasma flow. AcPAH would make a better test compound for the measurement of the renal plasma flow, as it is totally cleared via the kidney, it is not metabolised, and can be measured accurately and specifically using HPLC (Newman et al, 1949; Statius Van Eps, 1967).

The single injection methods described here for determining the GFR and ERPF, were used to establish the effects of tenoxicam, (a new non-steroidal anti-inflammatory drug) given for 10 days, on the renal function of healthy males. There was no significant changes, and, this is not entirely unexpected, as the GFR and RPF would not have been reduced in healthy subjects, unless they are sodium or fluid depleted (Carmichael & Shankel, 1985). Inhibition of the synthesis of renal prostaglandins only has an adverse effect when renal perfusion is reduced (Patrono & Dunn, 1987). To show such an effect with tenoxicam, studies should be carried out in "at risk groups" such as patients with renal disease and the elderly.

CONCLUSIONS

In these studies, the single intravenous injection of inulin and p-aminohippuric acid (PAH) have been investigated as a valid measure of the GFR and ERPF. The conclusions are summarized as follows:-

1) The total body and renal clearances of inulin decline progressively, two hours after a single intravenous bolus (70 mg/Kg) of inulin in 23 healthy male volunteers. The mean renal clearance fell from 97 ml/min/1.73 m^2 at 0-1 hours to 47 ml/ min/1.73 m^2 by 6-8 hours.

2) The total body clearance of inulin was significantly greater than the corresponding renal clearance (6 %) following single injection.

3) The total body and renal clearances of inulin were significantly lower at low, than high, steady state plasma concentrations.

4) The plot of the urinary excretion rate of inulin against its plasma concentration showed a negative deviation from the origin.

The results clearly show that at low plasma concentrations, the clearance of inulin is dependant on the plasma concentrations. It appears that there is low capacity saturable renal tubular reabsorption of inulin in addition to glomerular filtration, which becomes significant below plasma concentrations of 100 mg/l in normal subjects.

5) The total body and renal clearances of PAH fell after one hour, following a single intravenous injection of PAH (10 mg/Kg). The mean renal clearance of PAH fell

dramatically from 526 ml/min/1.73 m^2 at 0-1 hours to 241 ml/min/1.73 m^2 by 1-2 hours in 26 healthy male volunteers. Eighty two percent of the administered dose was recovered as PAH, and the remainder was recovered as its metabolite AcPAH.

6) The total body clearance of PAH was significantly greater than its corresponding renal clearance by 14 %, following single intravenous administration.

7) The renal clearance of PAH was significantly lower at low, than high, steady state plasma concentrations.

8) The metabolite of PAH, AcPAH was present in both plasma and urine, following intravenous administration of PAH during constant infusion studies and following a single injection. The renal clearance of AcPAH was significantly greater than that of the parent compound and was significantly greater at low, than high, plasma concentrations of PAH. The fractional urinary excretion of AcPAH decreased with increasing plasma concentrations of PAH.

The results clearly show that at low plasma concentrations, the renal clearance of PAH is highly dependant on the plasma concentration. There is evidence for saturable renal acetylation of PAH during the process of excretion. This effect significantly limits the renal clearance of PAH at plasma concentrations below 20 mg/l. The total body clearance of PAH is significantly greater than the renal clearance because of renal and extrarenal metabolism.

10) The single injection method for measuring the total body and renal clearances of inulin gave results

which were similar to those obtained by the standard constant infusion method in the same subjects when sampling was restricted to the first two hours following the single injection.

11) The renal clearance of PAH following a single injection was in the same order as that achieved during constant infusion in the same subjects, when sampling is restricted to the first hour.

12) These two single injection techniques were applied to the investigation of renal function during administration of tenoxicam, in 8 healthy hydrated males for 10 days. Tenoxicam had no significant effect on GFR or ERPF in these subjects

The single injection methods are limited by the depression of clearance at low plasma concentrations. However, if the sampling times are restricted, both the renal and total body clearances of inulin and the renal clearance of PAH, can be used as estimates of the GFR and RPF respectively. In the case of PAH, the renal clearance must be measured, but the total body clearance of inulin without urine collection, is a valid estimate of the GFR. The single injection methods are more convenient for the patient and investigator than the constant infusion methods and are ideal for single estimations of renal plasma flow and glomerular filtration rate.

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APPENDIX

APPENDIX I

DETAILS OF HEALTHY SUBJECTS STUDIED DURING CONSTANT INFUSION AND FOLLOWING SINGLE INJECTION OF p-AMINOHIPPURIC ACID (PAH) AND INULIN

			CONSTANT INFUSION		SINGLE INJECTION		DOSE ADMINISTERED SINGLE INJECTION	
SUBJECT	AGE (Y)	HEIGHT (M)	WEIGHT (KG)	S/A (M)	WEIGHT (KG)	S/A (M)	INULIN (MG)	PAH (MG)
RM	26	1,81	72,0	1,92	72,0	1,92	4870	715
LP	50	1,79	76,3	1,95	76,3	1,95	5020	704
GS	28	1,75	71,9	1,87	71,9	1,87	5188	674
JN	24	1,82	75,5	1,97	75,5	1,97	4990	727
DM	23	1,75	73,5	1,89	73,5	1,89	4840	710
MK	23	1,72	63,0	1,75	63,0	1,75	4807	643
CP	22	1,77	63,5	1,79	63,5	1,79	4850	581
BH	22	1,87	77,3	2,02	77,3	2,02	5320	712
BS	21	1,85	78,5	2,02	78,5	2,02	5201	715
PL	22	1,84	82,9	2,06	85,7	2,08	4967	-

S/A = Surface Area

		SINGLE INJECTION				
SUBJECT	AGE (Y)	WEIGHT (Kg)	HEIGHT (M)	S/A (M)	INULIN (MG)	PAH (MG)
PF	25	68,9	1,76	1,84	4838	628
GS 1	28	68,3	1,75	1,82	5032	-
JN 1	25	77,5	1,82	1,98	5249	-
TM	31	87,1	1,79	2,06	5280	812
GM	32	66,4	1,73	1,79	4927	647
AT	24	67,0	1,79	1,84	4918	636
EC	29	67,0	1,67	1,75	4631	640
WW	30	56,6	1,66	1,72	4677	548
AB	24	73,5	1,79	1,92	4970	720
RJ	27	57,2	1,65	1,62	4313	544
AD	29	65,4	1,71	1,77	4830	639
JA	29	88,5	1,87	2,14	5022	816
RF	24	76,9	1,73	1,92	5050	735
PD	22	67,7	1,74	1,81	4925	634
MS	25	74,8	1,73	1,88	5030	732
SA	24	67,5	1,72	1,79	4906	638
JG	29	70,4	1,79	1,88	5042	647
AH	28	74,9	1,87	1,99	-	719
SB	42	69,0	1,74	1,82	-	631

APPENDIX I.1a

INULIN (A) AND CREATININE (B) PLASMA CONCENTRATIONS DURING A CONSTANT INFUSION OF INULIN

A) INULIN

PLASMA CONCENTRATION (MG/L)

SUBJECT	BLANK	PERIOD (hours) 1	1,5	2	2,5	3
RM	<10	205	205	198	195	195
LP	<10	185	180	175	170	175
GS	<10	150	150	155	155	155
JN	<10	150	160	160	165	170
DM	<10	160	155	153	160	160
MK	<10	185	178	175	170	173
CP	<10	195	190	190	188	180
BH	<10	170	170	170	165	160
BS	<15	175	175	170	170	175
PL	<10	145	145	145	150	145

B) CREATININE

PLASMA CONCENTRATION (UMOL/L)

PERIOD (hours) 1	1,5	2	2,5	3
75	80	70	75	80
80	85	80	85	90
100	100	95	95	100
110	105	110	110	110
95	90	95	90	95
90	90	90	95	95
90	90	90	90	95
85	85	85	88	90
100	100	100	100	100
86	86	87	84	93

APPENDIX I.1b

p-AMINOHIPPURIC ACID (A) AND ACETYL p-AMINOHIPPURIC ACID (B) PLASMA CONCENTRATIONS DURING A CONSTANT INFUSION OF p-AMINOHIPPURIC ACID

A) p-AMINOHIPPURIC ACID

PLASMA CONCENTRATION (MG/L)

SUBJECT	BLANK	PERIOD (hours) 1	1,5	2	2,5	3
RM	0	24,95	27,84	27,66	27,61	27,53
LP	0	25,67	27,64	29,34	26,30	30,17
GS	0	27,67	28,35	28,68	31,30	29,94
JN	0	23,42	25,15	25,71	27,81	30,22
DM	0	24,92	25,77	28,05	29,2	30,21
MK	0	27,88	27,92	28,72	28,33	29,30
CP	0	33,60	35,40	37,50	37,54	35,41
BH	0	24,87	27,20	27,55	28,54	29,52
BS	0	26,06	29,33	34,21	33,40	33,41
RJ	0	24,80	28,31	28,40	29,40	29,60

B) ACETYL p-AMINOHIPPURIC ACID

PLASMA CONCENTRATION (MG/L)

PERIOD (hours) 1	1,5	2	2,5	3
3,40	3,88	4,18	3,89	3,67
2,32	3,03	3,29	3,39	3,34
2,30	2,54	2,77	2,93	2,94
2,49	2,88	3,17	3,24	3,33
2,01	2,35	2,24	2,57	2,62
2,87	3,33	3,26	3,46	3,22
3,08	3,47	3,56	3,39	3,40
2,71	2,95	3,21	3,16	3,10
3,10	4,08	4,33	4,46	4,41
2,57	2,82	2,88	2,99	3,06

APPENDIX I.2

RAW URINARY DATA RELATING TO INULIN, CREATININE, p-AINOHIPPURIC ACID (PAH) AND ACETYL-p-AMINOHIPPURIC ACID (AcPAH) DURING CONSTANT INFUSION STUDIES.

	COLLECTION PERIOD			URINARY CONCENTRATIONS			
SUBJECT	PERIOD hour	TIME mins	VOLUME ml	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
MK	1-1,5	29,60	275	2850	1600	1886,5	208,0
	1,5-2	30,00	270	2250	1200	1714,5	195,9
	2-2,5	29,60	134	3550	2050	2884,2	373,8
	2,5-3	29,95	245	2050	1200	1714,5	223,6
DM	1-1,5	30,38	231	2200	1650	1852,1	205,8
	1,5-2	30,32	200	2350	1700	2138,8	259,7
	2-2,5	30,43	122	4250	3000	4008,1	490,2
	2,5-3	30,50	215	2425	1550	2184,7	312,8
JN	1-1,5	29,25	226	1700	1850	1760,3	214,9
	1,5-2	29,58	110	3400	3150	3514,9	495,2
	2-2,5	30,07	102	4000	3600	4168,6	592,4
	2,5-3	29,67	146	2750	2400	2758,1	418,5
GC	1-1,5	29,33	206	1950	1900	1409,4	235,5
	1,5-2	29,83	240	1750	1500	1594,5	217,4
	2-2,5	29,67	130	3000	2550	2746,6	415,6
	2,5-3	29,67	134	2950	2500	2881,9	390,8
BH	1-1,5	29,42	143	2950	2300	2471,4	294,1
	1,5-2	29,60	146	3425	2850	3125,0	371,6
	2-2,5	29,67	192	2650	1900	2299,4	280,7
	2,5-3	33,01	180	2900	2150	2563,1	339,2
BS	1-1,5	29,37	170	3050	2500	2238,7	361,8
	1,5-2	29,92	108	4450	3250	3416,6	654,8
	2-2,5	29,60	180	2700	2100	2049,9	402,5
	2,5-3	29,55	206	2200	1700	1812,6	360,1
LP	1-1,5	29,40	282	1950	1050	1439,4	180,0
	1,5-2	29,93	263	2000	1000	1310,8	199,1
	2-2,5	29,93	136	3700	2050	3050,7	519,7
	2,5-3	29,96	144	3550	1950	2919,4	515,6
RM	1-1,5	30,00	234	2450	1500	1801,8	247,3
	1,5-2	30,00	211	2575	1460	2191,1	286,7
	2-2,5	30,50	356	1575	880	1347,6	140,6
	2,5-3	30,25	256	2200	1260	1791,8	256,8

APPENDIX I,2 CONTINUE

	COLLECTION PERIOD			URINARY CONCENTRATIONS			
SUBJECT	PERIOD hour	TIME mins	VOLUME ml	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
CP	1-1,5	29,50	66,5	7400	4600	5814,1	697,0
	1,5-2	29,57	206	2250	1400	1857,5	251,3
	2-2,5	29,75	206	2200	1400	1989,9	264,4
	2,5-3	29,80	210	2300	1400	1975,4	257,9
PL	1-1,5	30,47	46	12450	8480	-	-
	1,5-2	30,30	63	9150	6480	-	-
	2-2,5	30,18	151	3750	2680	-	-
	2,5-3	30,37	234	2300	1460	-	-
RJ	1-1,5	31,00	141	-	-	3066,7	424,2
	1,5-2	30,50	128	-	-	3499,0	511,7
	2-2,5	30,55	108	-	-	3579,9	606,8
	2,5-3	28,48	74	-	-	5253,5	869,8

APPENDIX I.3a

RAW INULIN PLASMA CONCENTRATIONS (MG/L) DATA FOLLOWING A SINGLE INJECTION OF INULN IN SUBJECTS WITH NORMAL RENAL FUNCTION

	SAMPLING TIME (MINS)																
SUBJECT	0	3	5	10	15	20	30	40	50	60	75	90	120	180	240	360	480
RM	<10	770	1220	790	610	488	423	253	220	203	160	128	100	80	55	33	25
LP	<10	345	850	775	600	480	360	200	215	195	160	140	110	70	53	45	25
GS	<10	305	670	640	495	460	390	335	285	253	205	170	125	83	65	35	20
JN	<10	220	590	550	430	350	265	260	225	200	170	155	125	85	60	35	5
DM	<10	460	750	660	470	385	310	240	210	195	160	140	110	95	80	40	20
MK	<10	220	570	555	460	410	340	270	240	200	173	145	113	70	53	25	15
CP	<10	365	770	760	600	500	373	305	260	233	193	165	128	53	60	33	20
BH	20	530	860	820	675	560	415	335	280	260	195	160	120	85	55	50	45
BS	<10	330	780	660	503	440	328	275	243	208	163	145	115	75	50	45	30
PL	35	340	690	650	415	375	300	230	195	165	135	115	90	55	45	25	15
GM	15	150	520	740	615	520	400	335	290	245	205	175	125	85	60	35	25
AT	<10	220	480	660	560	440	330	265	210	175	145	110	80	45	35	15	5
EC	5	700	1185	800	620	560	380	310	250	230	180	160	115	65	40	25	15
WW	<10	NT	1065	940	620	530	395	315	260	215	185	160	115	75	50	25	15
AB	5	860	960	820	660	530	365	280	225	200	155	135	100	55	35	25	9
RJ	10	350	690	660	540	470	350	280	220	185	135	125	90	65	45	30	20
JG	20	NT	795	740	640	560	335	270	220	185	150	125	100	60	40	20	20
JA	5	280	660	740	560	460	320	245	190	160	125	110	85	60	40	20	13
RF	10	250	645	720	580	480	365	275	230	185	150	125	95	60	40	25	25
PD	5	620	1050	880	680	550	365	290	245	215	185	145	110	70	45	30	25
MS	10	120	255	780	600	365	350	290	235	215	170	150	115	75	50	30	20
SA	5	310	900	680	540	450	325	275	235	205	165	135	100	60	35	25	20
AD	15	260	735	860	600	480	335	260	215	180	140	120	85	45	30	20	15
PF	10	200	660	740	580	440	275	200	170	155	130	115	75	40	20	15	10
GS 1	20	620	960	780	615	530	400	320	275	240	190	170	105	75	55	70	30
JN 1	25	1140	940	610	570	500	410	370	305	275	245	210	175	125	95	65	65
TM	10	360	630	680	540	450	315	255	195	170	145	120	90	55	35	20	25

APPENDIX I,3b
CREATININE PLASMA CONCENTRATION (UMOL/L) FOLLOWING A SINGLE INJECTION OF INULIN IN SUBJECTS WITH NORMAL RENAL FUNCTION

	SAMPLING TIMES (MINS)																
SUBJECT	0	3	5	10	15	20	30	40	50	60	75	90	120	180	240	360	480
RM	90	80	85	85	85	85	85	85	85	85	85	80	80	80	90	85	85
LP	90	85	80	85	85	80	80	80	80	85	80	80	80	80	80	85	85
GS	100	100	100	100	100	100	100	100	100	100	100	100	100	100	95	105	105
JN	105	110	110	105	105	105	105	110	105	105	105	105	105	105	100	115	105
DM	85	85	85	85	85	85	80	85	85	85	85	85	80	85	90	90	100
MK	95	90	90	90	90	90	95	90	90	90	90	90	95	95	95	100	105
CP	90	90	90	90	90	90	90	90	90	90	85	90	85	90	100	100	105
BH	90	90	95	95	90	95	90	90	90	90	90	90	90	95	90	100	100
BS	90	95	95	90	90	90	90	90	90	90	85	85	85	90	85	95	90
PL	101	98	97	89	96	84	89	87	91	86	82	77	79	81	95	91	92
GM	82	70	81	83	80	80	84	80	76	82	82	78	72	75	80	76	72
AT	90	87	87	83	87	85	86	85	83	90	92	88	91	92	80	97	97
EC	95	86	85	86	88	89	84	80	82	81	86	83	84	83	77	83	76
WW	96	NT	87	85	87	82	88	83	85	83	86	81	80	84	78	74	83
AB	86	85	80	80	82	85	90	87	95	82	82	84	78	80	85	90	89
RJ	96	100	97	95	92	92	93	90	86	94	90	91	87	86	84	99	90
AD	75	74	79	77	77	71	76	74	69	67	69	77	79	76	78	79	84
JA	88	81	77	78	81	80	78	82	74	87	83	80	82	84	87	77	71
RF	90	95	97	101	99	100	97	85	88	89	95	84	87	88	86	95	99
PD	88	82	84	85	76	79	77	86	82	87	80	83	86	86	79	83	84
MS	93	93	81	91	85	94	95	89	90	89	95	94	100	88	93	96	95
SA	72	74	73	72	74	79	100	75	77	70	81	78	77	74	81	85	85
JG	86	NT	85	81	78	78	78	69	69	74	76	69	73	76	72	69	72
PF	86	85	83	83	72	71	77	83	84	89	87	86	82	80	82	86	88
GS 1	95	95	95	95	90	90	95	95	95	95	95	90	95	95	90	100	100
JN 1	90	95	90	95	90	95	90	95	90	95	90	90	90	90	90	95	90
TM	89	89	83	78	83	82	80	90	83	78	83	85	81	77	86	83	86

APPENDIX I.3c

p-AMINOHIPPURIC ACID PLASMA CONCENTRATIONS (MG/L) FOLLOWING A SINGLE INJECTION OF p-AMINOHIPPURIC ACID IN SUBJECTS WITH NORMAL RENAL FUNCTION

	SAMPLING TIME (MINS)											
NAME	3	5	10	15	20	30	40	50	60	75	90	120
GM	21,76	31,89	28,76	24,72	18,56	13,43	8,85	6,62	4,84	3,75	2,74	1,47
AT	38,10	35,15	22,33	17,12	11,56	7,59	4,42	3,37	2,38	1,57	1,02	0,53
EC	54,87	44,18	26,09	18,03	13,05	7,97	6,00	3,68	3,27	1,82	1,47	1,05
WW	nt	41,79	27,48	17,23	14,35	8,89	6,40	4,77	3,39	2,45	1,90	1,13
AB	57,02	51,48	34,77	22,55	16,44	8,37	5,58	3,86	2,95	1,76	1,19	0,63
RJ	32,84	37,24	24,87	19,09	14,00	8,76	5,06	3,55	2,77	1,52	1,28	0,60
JG	33,28	37,71	22,25	16,08	11,00	6,60	4,29	3,13	2,33	1,63	0,95	0,71
AD	32,37	32,26	22,07	15,59	10,56	6,37	3,84	2,63	1,83	1,10	0,73	0,45
AH	63,10	41,50	24,16	15,09	11,47	8,87	5,87	4,62	3,49	2,80	2,11	0,81
SB	67,93	43,43	25,45	17,35	12,92	7,52	5,24	3,77	2,82	2,22	1,72	1,18
JA	60,90	38,80	26,56	19,96	12,25	8,84	5,15	4,26	3,06	1,74	1,65	0,96
PD	70,50	42,84	24,67	17,16	13,93	8,23	6,46	4,88	3,95	2,55	1,88	1,56
SA	40,70	39,58	26,75	16,82	13,91	8,80	5,67	4,02	3,24	2,39	1,74	1,09
RF	70,20	42,50	26,08	18,25	11,89	8,06	4,92	4,86	3,49	2,80	2,11	1,42
MS	46,98	34,44	30,10	20,91	15,56	10,18	6,23	4,64	4,11	2,95	2,75	1,80
PF	54,47	39,36	24,23	16,30	12,98	6,89	3,96	2,90	2,00	0,96	0,84	0,27
TM	51,97	42,24	27,09	18,95	14,11	9,14	6,59	4,50	3,50	2,77	1,96	1,17
MK	38,01	39,61	29,00	20,10	15,54	10,05	6,82	4,71	3,52	2,20	1,67	1,07
DM	52,69	37,79	24,18	16,80	12,70	7,72	5,42	4,36	3,46	2,40	1,75	1,09
JN	46,93	38,82	27,25	18,14	15,23	10,47	6,77	5,12	4,28	3,04	2,04	1,64
BH	49,33	50,12	34,22	20,06	15,54	8,43	5,87	4,08	3,56	2,35	1,56	0,97
BS	49,60	47,19	31,73	21,81	16,09	11,20	7,35	5,51	4,67	2,48	1,94	1,44
GS	52,49	39,29	30,28	20,12	15,86	9,60	6,65	4,75	3,54	2,72	1,90	1,21
LP	57,10	38,00	24,80	16,50	11,90	7,60	5,20	4,10	3,00	2,40	2,00	1,10
RM	66,80	47,10	26,00	18,90	15,20	10,60	6,90	4,90	4,40	3,00	2,30	1,80
CP	50,10	44,43	32,49	24,09	18,37	12,38	7,71	5,75	5,31	3,44	2,94	1,80

APPENDIX I,3d

ACETYL-p-AMINOHIPPURIC ACID PLASMA CONCENTRATIONS (MG/L) FOLLOWING A SINGLE INJECTION OF p-AMINOHIPPURIC ACID IN SUBJECTS WITH NORMAL RENAL FUNCTION

	SAMPLING TIME (MINS)											
NAME	3	5	10	15	20	30	40	50	60	75	90	120
GM	Trace	Trace	0,42	0,86	0,95	1,24	1,29	1,24	1,07	0,88	0,73	0,54
AT	1,16	1,25	1,45	1,49	1,50	1,37	1,19	1,01	0,83	0,58	0,46	0,22
EC	1,15	1,91	1,70	1,84	2,05	1,68	1,45	1,19	0,90	0,69	0,59	0,40
WW	NT	0,57	1,71	2,17	2,02	1,93	1,72	1,40	1,16	0,97	0,77	0,51
AB	1,55	1,57	1,69	1,97	1,77	1,61	1,29	1,00	0,86	0,60	0,51	0,32
RJ	0,40	0,56	1,01	1,25	1,31	1,34	1,23	0,98	0,88	0,64	0,53	0,34
JG	0,98	1,51	1,15	1,36	1,35	1,33	1,15	0,97	0,78	0,56	0,41	0,28
AD	1,27	1,40	1,68	1,52	1,50	1,33	1,07	0,87	0,70	0,50	0,37	0,23
AH	0,70	0,89	1,17	1,46	1,41	1,19	1,12	0,96	0,74	0,58	0,40	0,22
SB	0,79	0,92	1,28	1,50	1,51	1,62	1,43	1,22	1,06	0,88	0,75	0,59
JA	0,69	0,82	1,19	1,43	1,44	1,34	1,24	1,01	0,83	0,63	0,50	0,33
PD	0,84	1,05	1,38	2,15	2,07	1,96	1,85	1,70	1,43	1,20	0,94	0,78
SA	0,58	0,95	1,04	1,35	1,43	1,35	1,21	0,98	0,81	0,66	0,49	0,28
RF	1,23	1,23	1,71	2,02	2,08	1,79	1,51	1,35	1,07	0,92	0,72	0,46
MS	0,78	0,87	1,19	1,45	1,56	1,60	1,52	1,25	1,06	0,90	0,61	0,51
PF	0,88	0,96	1,11	1,36	1,27	1,13	0,82	0,71	0,62	0,45	0,37	0,25
TM	1,50	1,53	1,91	2,01	2,07	1,88	1,82	1,42	1,25	1,04	0,84	0,56
MK	0,44	0,59	1,04	1,36	1,45	1,57	1,41	1,39	1,13	0,95	0,74	0,54
DM	0,55	1,14	0,97	1,13	1,21	1,05	1,01	0,98	0,78	0,72	0,61	0,39
JN	1,12	0,86	1,33	1,52	1,48	1,86	1,49	1,36	1,26	0,98	0,72	0,61
BH	0,93	1,00	1,44	1,72	1,74	1,72	1,52	1,28	1,12	0,86	0,69	0,45
BS	0,97	1,27	1,97	2,34	2,43	2,16	1,93	1,67	1,43	1,20	0,97	0,72
GS	0,60	0,66	1,08	1,25	1,31	1,33	1,23	1,09	0,98	0,81	0,65	0,46
LP	1,17	1,28	1,74	1,78	2,05	1,81	1,51	1,29	1,05	0,85	0,72	0,47
RM	2,78	2,78	2,93	2,75	2,73	2,32	1,87	1,57	1,32	0,98	0,72	0,52
CP	1,03	0,60	0,95	1,10	1,50	1,31	1,37	1,26	1,10	1,02	0,91	0,65

APPENDIX I.4
RAW URINARY DATA CONCERNING INULIN, CREATININE, p-AMINOHIPPURIC ACID (PAH) AND ACETYL-p-AMINOHIPPURIC ACID (AcPAH) DURING SINGLE INJECTION STUDIES IN SUBJECTS WITH NORMAL RENAL FUNCTION

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
GM	0-1	105	71	21500	5860	4459	454
	1-2	590	60	1700	650	70	57
	2-3	370	60	1400	1410	26	45
	3-4	340	60	1000	1600	14	30
	4-6	710	120	550	1480	-	13
	6-8	340	120	500	2750	-	10
	8-24	800	960	185	10500	-	-
AT	0-1	482	80	5283	1686	1014	141
	1-2	285	60	3900	2380	89	141
	2-3	445	60	1100	1400	11	24
	3-4	375	60	690	1660	8	12
	4-6	365	120	705	3980	-	8
	6-8	770	120	210	1980	-	-
	8-24	780	960	645	13070	-	-
EC	0-1	570	68	4859	1285	857	118
	1-2	346	60	2700	1630	69	95
	2-3	326	60	1600	1730	22	48
	3-4	325	60	990	1720	11	25
	4-6	144	120	2100	7250	-	56
	6-8	34	120	1750	13800	-	38
	8-24	908	960	465	9410	-	-
WW	0-1	618	74	4130	946	710	106
	1-2	256	60	3200	1720	66	129
	2-3	350	60	1400	1340	17	42
	3-4	230	60	1300	1900	-	30
	4-6	360	120	750	2250	-	16
	6-8	480	120	350	1920	-	-
	8-24	935	960	250	6930		
AB	0-1	751	69	4133	1096	829	89
	1-2	330	60	2650	1850	73	99
	2-3	320	60	1600	1940	28	48
	3-4	345	60	850	1990	15	19
	4-6	238	120	1200	6020	-	20
	6-8	325	120	350	5000	-	7
	8-24	1144	960	50	8850	-	-
RJ	0-1	528	69	4259	1245	881	120
	1-2	405	60	1750	1360	37	77
	2-3	156	60	2200	3380	24	87
	3-4	309	60	900	1700	7	21
	4-6	384	120	735	3460	-	14
	6-8	159	120	765	7530	-	2
	8-24	1032	960	360	8840	-	-

APPENDIX I,4 CONTINUE

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
JG	0-1	732	79	3898	1012	741	89
	1-2	422	60	2700	1480	49	90
	2-3	295	60	1550	2100	17	52
	3-4	330	60	850	1800	11	20
	4-6	555	120	500	2120	-	8
	6-8	398	120	250	2960	-	-
	8-24	810	960	250	12060	-	-
AD	0-1	530	71	5906	1241	2060	149
	1-2	290	60	2950	1850	177	118
	2-3	295	60	1550	1960	49	93
	3-4	375	60	650	1500	15	37
	4-6	600	120	350	1850	7	12
	6-8	245	120	450	4340	-	-
	8-24	730	960	300	12270	-	-
AH	0-1	572	89	-	-	1021	126
	1-2	432	60	-	-	60	89
	2-3	296	60	-	-	17	62
	3-4	335	60	-	-	12	25
	4-6	195	120	-	-	-	35
	6-8	180	120	-	-	-	12
SB	0-1	152	76	-	-	3161	498
	1-2	158	60	-	-	122	222
	2-3	340	60	-	-	18	48
	3-4	260	60	-	-	7	32
	4-6	526	120	-	-	-	14
	6-8	150	120	-	-	-	19
JA	0-1	491	73	5511	1812	1406	139
	1-2	278	60	2950	2470	124	128
	2-3	354	60	1200	1960	28	41
	3-4	215	60	1050	3100	15	32
	4-6	572	120	500	2340	-	7
	6-8	530	120	315	2610	-	-
	8-24	898	960	195	6290	-	-
SA	0-1	691	72	3439	1030	788	101
	1-2	310	60	2600	1750	79	119
	2-3	354	60	1450	1840	17	52
	3-4	149	60	1600	3880	15	42
	4-6	430	120	555	2610	-	12
	6-8	340	120	420	3770	-	-
	8-24	1285	960	225	6640	-	-

APPENDIX I.4 CONTINUE

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
PD	0-1	460	72	5387	1334	1009	142
	1-2	290	60	4350	1490	79	105
	2-3	270	60	2450	2500	20	105
	3-4	218	60	1350	2520	26	53
	4-6	280	120	405	4190	-	40
	6-8	118	120	390	2900	-	-
	8-24	910	960	345	9430	-	-
RF	0-1	286	67	10098	5298	1922	275
	1-2	507	60	1750	1530	59	81
	2-3	214	60	2050	3490	26	88
	3-4	436	60	700	1770	-	22
	4-6	318	120	950	4660	-	23
	6-8	160	120	900	9630	-	21
	8-24	960	960	285	12750	-	-
MS	0-1	546	69	4797	1449	982	131
	1-2	334	60	2350	1990	72	105
	2-3	305	60	1550	2140	15	87
	3-4	298	60	950	2300	3	30
	4-6	395	113	650	3100	-	19
	6-8	615	131	300	2640	-	5
	8-24	620	949	480	15240	-	-
JN 1	0-1	550	91	4100	2050	-	-
	1-2	437	60	2100	1600	-	-
	2-3	408	60	1500	1700	-	-
	3-4	430	60	1000	1600	-	-
	4-6	381	120	1150	3950	-	-
	6-8	668	120	400	2250	-	-
	8-24	525	960	750	21000	-	-
GS 1	0-1	460	84	5500	2300	-	-
	1-2	445	60	2100	1500	-	-
	2-3	345	60	1300	1850	-	-
	3-4	307	60	960	2050	-	-
	4-6	180	120	1800	6850	-	-
	6-8	362	120	445	3550	-	-
	8-24	815	960	400	11500	-	-
PF	0-1	915	73	2898	1411	713	39
	1-2	328	60	2250	1790	160	90
	2-3	220	60	1750	3000	47	60
	3-4	143	60	1600	4290	12	40
	4-6	630	120	345	1830	7	32
	6-8	425	120	270	2600	-	4
	8-24	672	960	420	12140	-	-

APPENDIX I,4 CONTINUE

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
PL	0-1	1130	86	2612	1267	822	42
	1-2	415	60	2250	1730	312	129
	2-3	385	60	1400	1880	78	94
	3-4	374	60	915	2080	36	44
	4-6	480	120	675	2940	22	22
	6-8	315	120	585	5270	-	11
	8-24	518	960	630	19460	-	-
TM	0-1	774	90	3816	1369	960	60
	1-2	304	60	3450	2360	392	237
	2-3	350	60	1550	1850	102	149
	3-4	258	60	1250	2570	19	51
	4-6	500	120	700	2970	8	40
	6-8	238	120	600	6540	-	18
	8-24	1620	960	250	6920	-	-
DM	0-1	160	100	16000	7500	3119	506
	1-2	340	60	2900	2000	105	150
	2-3	275	60	1800	2550	36	81
	3-4	360	60	890	2000	22	27
	4-6	620	120	500	2450	-	13
	6-8	171	120	857	8300	-	-
	8-24	2240	960	174	4854	-	-
MK	0-1	560	ND	3400	1700	768	105
	1-2	425	60	2125	1600	161	84
	2-3	340	60	1600	2100	42	57
	3-4	202	60	1150	2900	2	34
	4-6	345	120	670	3850	-	20
	6-8	238	120	283	3200	-	6
	8-24	645	960	380	12884	-	-
CP	0-1	55	88	44500	19500	7646	1124
	1-2	100	60	9500	5900	344	503
	2-3	500	60	900	1200	14	55
	3-4	363	60	890	1600	2	37
	4-6	900	120	420	1600	-	12
	6-8	134	120	1075	8800	-	-
	8-24	601	960	406	14985	-	-
RM	0-1	193	ND	12900	5100	2624	311
	1-2	348	60	2850	1800	61	97
	2-3	344	60	1325	1700	12	43
	3-4	238	60	1475	2600	7	38
	4-8	262	240	1600	9200	-	24
	8-24	451	960	425		-	-

APPENDIX I,4 CONTINUE

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
LP	0-1	467	ND	5150	1800	1046	160
	1-2	426	60	2150	1250	81	99
	2-3	325	60	1550	1750	17	66
	3-4	94	60	2950	5700	13	89
	4-8	151	240	3300	15600	-	91
	8-24	1156	960	492	8006	-	-
BH	0-1	450	75	5400	2600	1119	133
	1-2	430	60	2300	1750	102	89
	2-3	360	60	1350	1850	20	49
	3-4	340	60	940	2050	9	28
	4-6	112	120	2800	11600	-	69
	6-8	186	120	900	8300	-	19
	8-24	4470	960	60	2750	-	-
BS	0-1	1068	100	2400	1350	471	56
	1-2	380	60	2575	2050	111	130
	2-3	260	60	1950	2700	29	85
	3-4	300	60	1080	2350	11	35
	4-6	202	120	1730	15200	20	42
	6-8	170	120	1050	9200	-	28
	8-24	3040	960	160	4450	-	-
GS	0-1	405	ND	5550	2900	1183	163
	1-2	555	60	1575	1350	76	69
	2-3	336	60	1250	2050	13	56
	3-4	280	60	825	2550	1	36
	4-8	735	240	600	3950	-	14
	8-24	746	960	372	15009	-	-
JN	0-1	430	ND	4450	3300	1177	169
	1-2	435	60	2050	1950	81	117
	2-3	345	60	1400	2000	19	64
	3-4	328	60	1050	2350	8	37
	4-8	1130	240	530	2900	-	15
	8-24	1530	960	323	7711	-	-

APPENDIX II

DETAILS OF SUBJECTS STUDIED USING REPEAT ESTIMATES OF INULIN SINGLE INJECTION METHOD

DAY			ONE			TWO			THREE		
SUBJECT	AGE (Y)	HEIGHT (M)	WEIGHT (KG)	S/A (M)	DOSE OF INULIN (MG)	WEIGHT (KG)	S/A (M)	DOSE OF INULIN (MG)	WEIGHT (KG)	S/A (M)	DOSE OF INULIN (MG)
GM	32	1,73	66,4	1,79	4927	67,4	1,92	5204	66,2	1,79	4953
EC	29	1,67	67,0	1,75	4631	67,6	1,95	4712	66,5	1,75	4598
AB	24	1,78	72,9	1,90	4930	72,5	1,87	5056	72,5	1,90	4882
RJ	27	1,65	57,2	1,62	4313	56,0	1,97	4310	56,9	1,62	4278
AD	29	1,71	65,4	1,77	4830	66,3	1,89	4793	66,8	1,78	4863
JA	29	1,87	88,5	2,14	5022	88,2	1,75	4918	89,0	2,14	4821
MS	25	1,73	74,8	1,88	5030	75,4	1,79	5100	76,8	1,92	5100
SA	24	1,72	67,5	1,79	4906	67,6	2,02	4942	68,0	1,80	4999

S/A = SURFACE AREA

APPENDIX II.1

INULIN PLASMA CONCENTRATIONS (MG/L) FOLLOWING REPEATED ESTIMATES OF THE INULIN SINGLE INJECTION METHOD

	TIME (Min)												
SUBJECT	0	3	5	10	15	20	30	40	50	60	75	90	120
GM 1	15	150	520	740	615	520	400	335	290	245	205	175	125
GM 2	20	300	560	720	600	500	380	325	280	250	210	180	135
GM 3	20	130	390	760	820	540	400	325	280	235	185	150	115
EM 1	5	700	1185	800	620	560	380	310	250	230	180	160	115
EM 2	15	710	855	860	680	550	375	315	265	220	190	155	120
EM 3	15	700	1035	840	680	550	395	325	265	240	200	175	130
AB 1	20	490	840	780	600	440	295	235	195	160	130	110	85
AB 2	10	700	750	860	700	500	360	295	255	205	175	145	110
AB 3	15	700	1080	780	600	480	325	270	230	190	160	135	95
RJ 1	10	350	690	660	540	470	350	280	220	185	135	125	90
RJ 2	5	690	1035	700	580	540	375	295	230	185	145	130	105
RJ 3	5	330	750	620	520	470	360	270	205	170	130	115	95
AD 1	15	260	735	860	600	480	335	260	215	180	140	120	85
AD 2	10	480	795	760	560	450	320	250	200	175	140	120	85
AD 3	5	330	645	720	600	470	320	255	210	180	145	115	85
JA 1	5	280	660	740	560	460	320	245	190	160	125	110	90
JA 2	2,5	350	690	600	500	460	315	255	195	160	120	85	60
JA 3	5	190	450	640	500	400	280	220	180	155	125	125	110
SA 1	5	310	900	680	540	450	325	275	235	205	165	135	100
SA 2	15	500	765	740	540	490	345	275	230	205	165	140	95
SA 3	5	290	600	660	540	440	335	265	210	180	155	120	90
MS 1	10	120	255	780	600	365	350	290	235	215	170	150	115
MS 2	5	390	615	720	620	520	355	275	235	205	160	140	100
MS 3	5	330	585	740	600	490	335	270	225	195	165	140	105

1 = Day one, 2 = Day two, 3 = Day three

APPENDIX II.2

INULIN URINARY DATA FROM REPEATED ESTIMATES OF INULIN SINGLE INJECTION METHOD

	DAY	ONE			TWO			THREE		
SUBJECT	PERIOD	TIME min	VOLUME ml	INULIN mg/l	TIME min	VOLUME ml	INULIN mg/l	TIME min	VOLUME ml	INULIN mg/l
GM	0-1	71	105	21500	67	615	4500	51	594	4050
	1-2	60	590	1700	60	445	2150	60	340	2850
EC	0-1	68	570	4859	66	670	5781	70	612	3876
	1-2	60	346	2700	60	495	1850	60	315	2850
AB	0-1	90	652	3657	69	670	3112	67	310	5276
	1-2	60	290	3100	60	495	2400	60	237	4200
RJ	0-1	69	528	4259	65	407	4975	69	653	3391
	1-2	60	405	1750	60	235	2550	60	237	2550
AD	0-1	71	530	5906	71	169	20077	69	448	8500
	1-2	60	290	2950	60	315	2800	60	268	3350
JA	0-1	73	491	5511	66	382	7113	70	479	5424
	1-2	60	278	2950	60	140	4900	60	270	2800
SA	0-1	72	691	3439	66	750	3384	70	609	3588
	1-2	60	310	2600	60	494	1650	60	460	1750
MS	0-1	69	546	4796	67	404	6500	73	344	7341
	1-2	60	334	2350	60	540	1300	60	345	2400

APPENDIX III

SUBJECT DETAILS OF PATIENTS WITH RENAL IMPAIRMENT

SUBJECT	AGE (Y)	WEIGHT (Kg)	HEIGHT (M)	S/A (M^2)	DOSE OF INULIN (MG)
JB	46	76,7	1,76	1,92	5070
EC	63	86,3	1,74	2,00	5108
AD	61	74,0	1,61	1,78	5124
EH	65	78,8	1,67	1,87	5120
WA	65	64,0	1,70	1,74	5048
JH	65	61,0	1,68	1,75	5081
CP	56	87,5	1,81	2,08	5101

S/A = SURFACE AREA

APPENDIX III.1

INULIN AND CREATININE PLASMA CONCENTRATIONS FOLLOWING A SINGLE INJECTION OF INULIN IN PATIENTS WITH RENAL IMPAIRMENT

INULIN PLASMA CONCENTRATION MG/L

		TIME (MINUTES)												
SUBJECT	BLANK	5	10	15	20	30	45	60	90	120	180	240	360	480
JB	<10	1000	940	780	690	600	505	460	390	345	295	255	205	155
EC	20	660	740	680	570	520	430	390	355	320	270	240	190	160
AD	<10	NM	960	840	740	620	470	425	340	310	240	195	145	120
EH	<10	NM	920	800	700	570	525	430	380	340	295	245	200	160
EB	40	975	1050	870	645	600	450	405	320	280	225	185	130	105
JH	7	NM	990	795	675	525	420	330	265	210	150	115	75	50
WA	14	NM	1050	810	690	555	420	345	260	225	145	125	85	60
CP	26	NM	720	630	555	450	345	300	250	220	165	135	95	75

NM = NOT MEASURED

CREATININE PLASMA CONCENTRATIONS UMOL/L

	TIME (MINUTES)							
SUBJECT	BLANK	60	90	120	180	240	360	480
JB	302	294	292	283	285	290	293	294
EC	264	263	262	255	243	249	250	253
AD	158	155	153	151	143	146	158	150
EH	243	232	233	233	231	230	227	238
EB	297	296	296	292	295	293	292	294
JH	131	126	125	120	139	123	124	125
WA	131	122	127	118	125	124	125	132
CP	189	180	179	180	178	182	182	187

APPENDIX III,2
INULIN AND CREATININE URINARY DATA FOLLOWING A SINGLE INJECTION OF INULIN IN PATIENTS WITH RENAL IMPAIRMENT

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l
JB	0-1	255	66	3550	3080
	1-2	235	60	2700	2680
	2-3	286	61	1700	2290
	3-4	265	60	1450	2290
	4-6	365	121	1600	3310
	6-8	336	121	1300	3850
EC	0-1	172	69	3600	4090
	1-2	254	60	2400	2310
	2-3	252	60	1750	2070
	3-4	272	61	1450	1970
	4-6	336	118	1600	2660
	6-8	465	120	1200	2520
AD	0-1	492	0	2800	970
	1-2	342	0	2450	1090
	2-3	304	0	1950	1210
	3-4	245	0	1750	1350
	4-6	360	0	1800	2010
	6-8	410	0	1150	1980
EH	0-1	390	70	3150	1770
	1-2	347	61	200	1700
	2-3	370	60	1500	1560
	3-4	325	61	1350	1770
	4-6	336	120	1850	3090
	6-8	355	119	1450	3100
EB	0-1	474	69	2650	920
	1-2	416	60	1750	970
	2-3	262	60	1250	1070
	3-4	250	61	1200	1110
	4-6	536	120	1150	1500
	6-8	534	120	750	1570
JH	0-1	543	73	3800	1140
	1-2	342	60	2600	1440
	2-3	350	60	1600	1390
	3-4	296	59	1250	1610
	4-6	262	121	1800	3640
	6-8	280	120	1000	3310

APPENDIX III.2 CONTINUE

SUBJECT	PERIOD	VOLUME ml	TIME min	INULIN mg/l	CREATININE umol/l
WA	0-1	480	76	3700	1240
	1-2	336	59	2450	1170
	2-3	406	60	1500	1130
	3-4	344	60	1250	1240
	4-6	450	119	1250	2020
	6-8	380	122	1000	2430
CP	0-1	420	68	3450	2220
	1-2	465	74	1750	1790
	2-3	290	48	1250	1880
	3-4	224	58	950	1840
	4-6	560	122	1100	2880
	6-8	738	120	500	2080

APPENDIX IV

DETAILS OF SUBJECTS INVOLVED IN THE STEP UP AND STEP DOWN CONSTANT INFUSION INCLUDING, AGE, HEIGHT, SURFACE AREA (S/A),AND INFUSION CONCENTRATIONS.

	STEP UP INFUSION						STEP DOWN INFUSION			
					INFUSION CONCENTRATION				INFUSION CONCENTRATION	
SUBJECT	AGE (Y)	HEIGHT (M)	WEIGHT (KG)	S/A (M)	INULIN (MG/%)	PAH (MG)	WEIGHT (KG)	S/A (M)	INULIN (MG/%)	PAH (MG)
JN	26	1,82	77,2	1,97	1500	12776	77,4	1,97	1550	12469
GS	29	1,75	70,1	1,84	1750	12897	71,0	1,86	1500	13128
SB	42	1,74	71,6	1,85	1679	12669	71,6	1,85	1600	10227
AD	30	1,71	64,5	1,75	1650	12323	64,9	1,75	1650	12036
SM	28	1,64	55,2	1,59	1650	12994	55,6	1,59	1650	12347
WW	27	1,86	75,1	1,98	1600	12707	73,9	1,97	1650	12203
BB	36	1,62	69,0	1,73	1950	12179	68,5	1,73	1650	11724
BW	25	1,72	70,1	1,82	1650	12807	69,0	1,81	1500	12162

APPENDIX IV.1a
INULIN PLASMA CONCENTRATIONS DURING STEP UP AND DOWN CONSTANT INFUSIONS OF INULIN

INULIN PLASMA CONCENTRATIONS (MG/L) ACHIEVIED DURING THE STEP UP INFUSION

	TIME (hours)												
SUBJECT	0	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	<10	25	25	23	23	60	55	50	45	130	115	105	100
GS	<10	35	40	35	40	75	75	75	75	210	190	185	185
SB	<10	25	25	30	25	65	60	55	60	145	145	140	140
BB	<10	35	40	43	35	80	65	65	75	195	173	170	170
AD	<10	30	30	30	30	60	55	55	55	185	170	165	160
WW	10	50	40	40	40	75	65	60	60	170	153	153	155
SM	10	45	45	45	45	85	85	85	80	235	210	210	205
BW	15	40	38	38	38	85	75	75	70	230	190	185	185

INULIN PLASMA CONCENTRATIONS (MG/L) ACHIEVIED DURING THE STEP DOWN INFUSION

	TIME (hours)												
SUBJECT	0	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	<10	215	200	195	190	145	125	110	105	85	75	80	55
GS	<10	220	200	190	175	130	100	100	110	75	80	80	60
SB	<10	165	155	150	155	120	95	85	80	75	65	60	55
BB	10	215	185	185	175	140	110	105	95	75	70	65	65
AD	<10	195	180	175	175	115	100	95	90	65	55	50	35
WW	<10	175	150	150	150	105	90	85	75	55	45	45	45
SM	10	300	265	250	235	170	145	130	125	105	95	90	85
BW	<10	230	230	190	185	130	115	105	100	85	75	70	6

APPENDIX IV.1b
CREATININE PLASMA CONCENTRATIONS DURING STEP UP AND DOWN CONSTANT INFUSIONS OF INULIN

CREATININE PLASMA CONCENTRATIONS (UMOL/L) DURING THE STEP UP INFUSION

	TIME (hours)												
SUBJECT	0	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	107	103	101	103	99	95	90	99	97	100	97	97	93
GS	93	100	89	87	93	92	97	92	91	93	96	91	91
SB	71	60	67	68	63	67	63	67	68	56	58	72	72
AD	77	78	81	79	75	75	71	76	69	74	75	80	83
BB	73	74	86	73	79	70	65	67	77	72	72	74	73
WW	79	78	76	76	73	72	75	75	74	68	73	74	75
SM	76	88	90	85	85	88	81	84	73	78	71	102	98
BW	79	85	87	82	80	78	78	76	78	78	77	73	80

CREATININE PLASMA CONCENTRATIONS (UMOL/L) DURING THE STEP DOWN INFUSION

	TIME (hours)												
SUBJECT	0	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	107	103	101	103	99	95	90	99	97	100	97	97	93
GS	85	83	71	77	84	80	76	89	90	88	91	89	83
SB	47	56	58	57	58	53	55	55	56	59	59	55	58
BB	81	76	73	81	74	76	75	78	77	75	73	73	74
AD	68	78	85	81	86	78	78	78	74	80	82	79	81
WW	69	70	72	69	70	70	69	66	65	71	72	71	71
SM	81	83	80	79	79	79	76	77	79	79	76	74	75
BW	80	81	81	85	76	83	88	76	85	88	71	79	77

APPENDIX IV,2c
RAW p-AMINOHIPPURIC ACID PLASMA CONCENTRATIONS FOR THE STEP UP AND DOWN CONSTANT INFUSIONS OF
OF p-AMINOHIPPURIC ACID

PAH PLASMA CONCENTRATIONS (MG/L) STEP UP INFUSION

	TIME (hours)											
SUBJECT	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	3,70	3,60	4,00	4,00	9,30	8,04	8,35	8,16	26,13	25,30	25,40	25,80
GS	4,43	4,33	4,28	4,28	10,60	9,14	9,16	9,22	26,04	25,41	25,35	25,05
SB	4,01	3,71	3,93	3,93	8,72	7,44	7,79	7,67	21,44	21,83	22,82	22,63
AD	3,68	5,34	3,73	3,73	8,24	6,90	6,69	7,03	24,40	21,96	22,43	21,22
BB	4,90	4,40	4,50	4,50	11,20	9,71	9,17	9,35	28,10	26,70	27,75	29,90
SM	6,48	5,99	6,03	6,03	13,80	12,21	12,45	12,60	39,05	35,51	34,14	33,75
WW	2,99	3,01	3,82	3,82	6,33	6,01	5,07	4,99	16,41	15,11	18,19	16,45
BW	5,65	4,50	6,55	6,55	11,07	9,38	8,83	8,71	31,50	25,10	24,29	28,78

PAH PLASMA CONCENTRATIONS (MG/L) STEP DOWN INFUSION

	TIME (hours)											
SUBJECT	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	20,95	21,60	22,30	23,69	11,30	9,80	8,98	8,49	5,09	4,56	4,77	4,34
GS	24,39	25,77	26,35	27,11	13,15	12,09	9,87	9,93	6,00	5,98	5,51	5,26
SB	20,84	22,63	21,04	21,83	11,75	9,83	9,58	9,67	5,89	5,50	4,80	4,80
AD	19,37	22,61	21,13	20,79	9,44	9,15	8,40	7,03	4,95	4,82	4,02	4,07
BB	23,24	22,70	24,90	24,70	11,20	10,62	9,50	9,50	5,60	5,60	5,00	5,40
SM	29,58	30,10	31,40	31,14	15,66	14,47	12,91	12,99	8,10	7,21	7,21	7,05
WW	14,51	13,15	14,70	15,06	7,99	6,48	5,94	5,94	3,21	2,87	2,93	2,85
BW	20,14	20,14	20,50	22,48	12,05	10,43	9,72	9,72	8,64	5,40	5,58	5,40

APPENDIX IV.1d

ACETYL-p-AMINOHIPPURIC ACID PLASMA CONCENTRATIONS DURING STEP UP AND DOWN CONSTANT INFUSIONS OF p-AMINOHIPPURIC ACID

AcPAH PLASMA CONCENTRATION (MG/L) STEP UP INFUSION

	TIME (hours)											
SUBJECT	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	1,06	1,12	1,17	1,11	1,95	1,89	1,83	1,81	3,16	3,55	3,56	3,45
GS	0,62	0,62	0,59	0,60	1,12	1,01	0,93	0,97	1,94	1,87	2,00	2,01
SB	0,69	0,70	0,69	0,71	1,34	1,25	1,14	1,13	2,87	2,83	2,81	2,74
AD	0,56	0,57	0,53	0,61	1,08	1,02	0,91	1,01	2,11	2,10	2,35	2,12
BB	0,66	0,74	0,62	0,74	1,40	1,42	1,39	1,53	2,75	3,02	3,29	3,22
SM	1,02	0,97	0,96	0,96	1,66	1,62	1,64	1,59	3,21	3,62	3,56	3,58
WW	0,40	0,38	0,36	0,33	0,67	0,55	0,60	0,52	1,55	1,36	1,19	1,26
BW	0,44	0,46	0,47	0,62	1,00	1,01	0,95	0,95	1,71	2,20	2,20	2,24

AcPAH PLASMA CONCENTRATIONS (MG/L) STEP DOWN INFUSION

	TIME (hours)											
SUBJECT	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	6,5
JN	3,99	3,95	4,20	4,22	3,03	2,56	2,16	1,88	1,51	1,23	1,08	0,89
GS	3,10	3,30	3,38	3,41	2,57	2,10	1,65	1,82	1,34	1,12	0,98	0,86
SB	3,00	3,24	3,22	3,22	2,50	1,99	1,70	1,53	1,23	1,18	0,91	0,81
AD	2,60	2,69	2,74	2,56	1,86	1,48	1,23	1,34	0,95	0,82	0,83	0,64
BB	2,63	2,80	2,78	3,07	2,24	1,84	1,69	1,47	1,25	1,05	0,97	0,92
SM	3,32	3,49	3,47	3,47	2,75	2,10	2,05	1,86	1,48	1,23	1,20	1,10
WW	1,67	1,59	1,47	1,48	1,01	0,79	0,85	0,74	0,55	0,45	0,48	0,38
BW	2,32	2,41	2,48	2,64	2,14	1,83	1,61	1,48	1,17	1,13	0,99	0,88

APPENDIX IV.2

RAW URINARY CONCENTRATIONS OF INULIN, CREATININE, p-AMINOHIPPURIC ACID AND ACETYL-p-AMINOHIPPURIC ACID DURING STEP UP AND DOWN CONSTANT INFUSIONS

STEP DOWN INFUSION STUDY

	COLLECTION PERIOD			URINARY CONCENTRATIONS			
SUBJECT	PERIOD hour	TIME mins	VOLUME ml	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
JN	1,5-2	28,8	292	2050	1340	1109,4	173,9
	2-2,5	29,5	268	2200	1460	984,2	157,8
	3,5-4	32,0	159	2400	2570	847,9	280,9
	4-4,5	30,5	210	1470	1940	600,9	192,7
	5,5-6	30,5	310	615	1280	193,3	132,0
	6-6,5	29,5	296	585	1250	192,2	199,2
GS	1,5-2	29,5	245	2300	1490	1356,4	199,9
	2-2,5	30,5	241	2400	1590	1490,2	227,4
	3,5-4	29,0	284	990	1370	448,9	138,5
	4-4,5	29,5	270	1035	1340	430,8	127,7
	5,5-6	29,0	242	720	1440	234,8	112,6
	6-6,5	29,5	195	810	1910	271,3	137,8
SB	1,5-2	31,2	178	4750	2070	2407,4	365,0
	2-2,5	29,5	69	5000	2320	2872,1	629,5
	3,5-4	29,6	135	2500	1930	798,2	228,7
	4-4,5	29,8	152	2600	2160	865,1	286,5
	5,5-6	29,8	210	1150	1630	306,4	159,1
	6-6,5	29,7	240	800	1300	213,5	157,7
AD	1,5-2	29,7	210	3300	1520	1891,0	289,7
	2-2,5	30,4	290	2300	1240	1280,2	218,4
	3,5-4	29,9	265	1450	1250	488,6	168,6
	4-4,5	29,3	240	1250	1340	491,1	158,2
	5,5-6	29,9	279	650	1210	196,6	101,9
	6-6,5	28,4	225	650	1360	222,2	121,4
BB	1,5-2	29,5	235	2650	1210	1492,5	229,8
	2-2,5	29,9	330	2100	920	1215,4	191,1
	3,5-4	29,8	134	2550	2060	933,5	262,7
	4-4,5	29,9	282	1380	1300	581,7	148,8
	5,5-6	30,2	259	690	1150	162,4	71,7
	6-6,5	30,1	253	660	1130	247,9	108,5

APPENDIX IV.2 CONTINUE

STEP DOWN INFUSION STUDY

	COLLECTION PERIOD			URINARY CONCENTRATIONS			
SUBJECT	PERIOD hour	TIME mins	VOLUME ml	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
SM	1,5-2	29,9	280	2300	960	1290,4	198,6
	2-2,5	30,4	240	2350	1180	1523,1	165,6
	3,5-4	29,9	215	1480	1320	635,3	187,4
	4-4,5	30,4	200	1375	1390	634,1	152,6
	5,5-6	30,5	255	700	1180	263,0	108,1
	6-6,5	30,4	255	650	1030	253,2	106,8
WW	1,5-2	29,6	275	2000	1070	1083,5	121,9
	2-2,5	29,6	451	1900	1040	1063,9	121,4
	3,5-4	29,4	370	1500	1330	584,5	136,3
	4-4,5	29,4	355	1200	1120	482,2	116,3
	5,5-6	29,5	342	525	930	164,5	60,2
	6-6,5	30,6	420	500	880	161,1	56,4
BW	1,5-2	29,9	210	3350	1330	1765,4	240,5
	2-2,5	30,2	243	3150	1370	1489,3	215,2
	3,5-4	29,6	203	1850	1560	594,6	173,7
	4-4,5	29,6	160	2100	2280	727,2	229,1
	5,5-6	29,8	152	1500	2000	273,6	203,8
	6-6,5	29,5	208	800	1440	269,1	144,5

STEP UP INFUSION STUDY

	COLLECTION PERIOD			URINARY CONCENTRATIONS			
SUBJECT	PERIOD hour	TIME mins	VOLUME ml	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
JN	1,5-2	29,5	170	450	2240	288,3	145,3
	2-2,5	29,5	256	315	1580	192,2	93,9
	3,5-4	29,0	186	920	2100	548,7	179,7
	4-4,5	28,0	263	640	1540	391,7	121,2
	5,5-6	29,0	256	1950	1470	1283,4	213,5
	6-6,5	28,0	244	1800	1510	1332,1	223,9
GS	1,5-2	30,0	263	345	1550	214,6	90,0
	2-2,5	29,7	218	405	1800	248,0	105,2
	3,5-4	29,7	260	680	1500	480,4	113,0
	4-4,5	29,6	255	660	1510	473,9	113,5
	5,5-6	29,5	283	2150	1350	1353,3	151,4
	6-6,5	28,5	212	2400	1740	1706,9	197,1

APPENDIX IV.2 CONTINUE

STEP UP INFUSION STUDY

	COLLECTION PERIOD			URINARY CONCENTRATIONS			
SUBJECT	PERIOD hour	TIME mins	VOLUME ml	INULIN mg/l	CREATININE umol/l	PAH mg/l	AcPAH mg/l
SB	1,5-2	29,7	221	400	1380	227,8	173,6
	2-2,5	29,8	350	225	750	128,0	99,2
	3,5-4	30,4	40	2850	4440	1953,4	566,8
	4-4,5	30,0	225	1100	1630	604,6	277,6
	5,5-6	29,7	390	1450	770	944,4	191,5
	6-6,5	30,3	185	2350	1420	1836,8	395,3
AD	1,5-2	29,5	270	420	1180	209,7	101,6
	2-2,5	29,4	174	600	1530	281,0	145,4
	3,5-4	30,4	322	750	1100	385,1	129,9
	4-4,5	29,9	233	945	1520	513,5	172,9
	5,5-6	30,6	148	4450	2340	2913,2	440,2
	6-6,5	29,7	252	2650	1300	1641,7	266,9
BB	1,5-2	29,8	340	345	970	187,2	75,2
	2-2,5	29,8	318	300	810	178,7	71,2
	3,5-4	29,5	270	720	1010	469,7	159,1
	4-4,5	29,9	295	645	870	397,4	102,4
	5,5-6	29,4	298	2100	900	1268,4	196,9
	6-6,5	30,2	295	2050	950	1266,0	206,8
SM	1,5-2	30,3	270	375	1480	222,1	97,9
	2-2,5	30,1	198	500	2070	291,3	108,8
	3,5-4	30,3	208	950	1920	593,8	180,9
	4-4,5	29,8	210	950	1380	590,1	180,6
	5,5-6	28,7	190	3050	1520	2022,6	305,3
	6-6,5	29,3	205	2750	1420	1890,1	303,2
WW	1,5-2,5	60,6	700	275	1150	157,4	77,9
	3,5-4,5	59,7	532	725	1550	472,4	131,4
	5,5-6,5	62,0	496	2175	1570	1633,3	210,2
BW	1,5-2	31,4	258	400	1170	213,9	86,9
	2-2,5	30,7	137	700	2550	454,7	175,9
	3,5-4	29,9	195	1050	1660	594,6	157,3
	4-4,5	30,5	105	1950	2970	1213,1	306,2
	5,5-6	29,8	294	2400	1180	1577,6	203,3
	6-6,5	29,5	234	2650	1410	1688,1	241,5

PUBLICATIONS

The following are the titles of published abstracts of papers presented to the British Pharmacological Society, which contained data included in this thesis.

FREESTONE, S., McAUSLANE, J.A.N., MACKAY, I.G., COWIE, J., WATSON, M.L., and PRESCOTT, L.F. (1986). Comparison of total body clearance and renal clearance of inulin and p-aminohippuric acid during constant infusion. British Journal of Clinical Pharmacology, **21**, 96P - 97P.

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